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(54) Title: ANGIOGENICALLY EFFECTIVE UNIT DOSE OF FGF–2 AND METHOD OF USE

(57) Abstract

The present invention has multiple aspects. In particular, in one aspect, the present invention is directed to a unit dose compositi n comprising 0.2 μg/kg to 48 μg/kg of an FGF–2 of SEQ ID NO: 2, or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In another aspect, the present invention is directed to a method for treating a human patient for coronary artery disease, comprising administering into one or more coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease a safe and angiogenically effective dose of a recombinant FGF–2, or an angiogenically active fragment or mutein thereof. The single unit dose composition of the present invention provides an angiogenic effect in a human CAD patient that lasts six months before re–treatment is required. In another aspect, the present invention is directed to a method of administration which optimizes patient's safety. In this embodiment, fluids, heparin and/or rate of infusion all play a role. In another aspect, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of FGF–2, alone or in combination with heparin, in a therapeutically effective carrier. The magnitude and duration of benefit were unexpected; in addition benefit with the IV route was unexpected.

Many of the various FGF molecules have been isolated and administered to various animal models of myocardial ischemia with varying and often times opposite results. According to Battler *et al.*, "the canine model of myocardial ischemia has been criticized because of the abundance of naturally occurring collateral circulation, as opposed to the porcine model, which 'excels' in its relative paucity of natural collateral circulation and its resemblance to the human coronary circulation." Battler *et al.*, *"Intracoronary Injection of Basic Fibroblast Growth Factor Enhances Angiogenesis in Infarcted Swine Myocardium," JACC, 22(7)*: 2001-6 (Dec. 1993) at page 2002, col.1. However, Battler *et al.*, who administered bovine bFGF (*i.e.*, FGF-2) to pigs in a myocardial infarct model, considered the varying results that are obtained from one animal species to another, and expressly discloses that the divergent results "thus emphasiz[e] the caution that must be exercised in extrapolating results from different animal models." Battler *et al.*, at page 2005, col.1. Further, Battler points out that "the dosage and mode of administration of bFGF [i.e., bovine FGF-2] may have profound implications for the biologic effect achieved." Battler, *et al.*, at page 2005, col.1. Thus, it is a further object of this invention to discover a dosage and a mode of administration of a fibroblast growth factor that would provide for the safe and efficacious treatment of CAD and/or post MI injury in a human patient. More generally, it is an object of the present invention to provide a pharmaceutical composition and method for inducing angiogenesis in a human heart.

In another aspect, the present invention is directed to a method of treating a human patient for CAD or to induce coronary angiogenesis therein. The method comprises administering into one or more coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease (or in need of angiogenesis) a safe and therapeutically effective amount of a recombinant FGF-2 (rFGF-2) or an angiogenically active fragment or mutein thereof. Typically, a portion of the safe and therapeutically effective amount is administered to each of two coronary vessels. The safe and therapeutically effective amount comprises about 0.2 μg/kg to about 48 μg/kg, of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In other embodiments, the safe and therapeutically effective amount comprises 0.2 μg/kg to 2 μg/kg, >2 μg/kg to <24 μg/kg, or 24 μg/kg to 48 μg/kg of rFGF-2 an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In absolute terms, the amount of rFGF-2 or angiogenically active fragment or mutein thereof that is used in the above method comprises .008 mg to 7.2 mg, more typically 0.3 mg to 3.5 mg, of rFGF-2 or an angiogenically active fragment or mutein thereof.

Because FGF-2 is a glycosoaminoglycan (*e.g.*, heparin) binding protein and the presence of a glycosoaminoglycan optimizes activity and AUC (see Figs. 3 and 4), the IC dosages of RFGF-2 of the present invention typically are administered from 0-30 minutes prior to the administration of a glycosoaminoglycan, such as a heparin. The heparin is administered IC or IV, typically IV. Optionally, the heparin is combined with the unit dose composition.

Because rFGF-2 releases nitric oxide which is a potent vasodilator, aggressive fluid management prior to (proactively) and during the infusion is critical to patient's safety. Administration of IV fluids (e.g., 500-1000 mL of normal saline) to establish a wedge pressure of 12 mm Hg prior to infusion and administration of boluses of IV fluids (e.g., 200 mL normal saline) for decreases of systolic blood pressure (e.g., <90 mm Hg) associated with infusion optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

Because EDTA is a potent chelator of calcium which is required for normal myocardial contraction and cardiac conduction, minimizing the

significant manner for all dosage ranges whether administered IC or IV. (Tables 2-6). In particular, the five scales assessed by the SAQ are exertional capacity, angina stability, angina frequency, treatment satisfaction, and disease perception. Relative to the baseline, the mean score for exertional capacity increased by 10.9 to 20.2 at 2 months; and by 16.5 to 24.1 at 6 months. For angina stability, the mean score increased by 32.1 to 46.2 at 2 months; and by 16.7 to 23.2 at 6 months. For angina frequency, the mean score increased by 20.0 to 32.9 at 2 months; and by 11.4 to 36.7 at 6 months. For treatment satisfaction, the mean score increased by 8.5 to 19.8 at 2 months; and by 6.3 to 19.8 at 6 months. For disease perception, the mean score increased by 20.2 to 27.8 at 2 months; and by 23.8 to 34.0 at 6 months. Generally, a change of 8 points on any scale is considered clinically significant. Thus, the observed changes of 8.5-46.2 are clinically significant for each of the five scales that were assessed. Even assuming a placebo effect whereby a mean change from baseline of 14 points is considered clinically significant, the results still provide for an unexpectedly superior effect at almost all scales that were assessed.

As part of this study, MRI was also performed on 33 human patients diagnosed with CAD to assess the effect of administering a single unit dose of rFGF-2 on their cardiac ejection fraction, regional myocardial function and perfusion (delayed arrival zone). Specifically, the patients were administered a single unit dose of 0.33 μg/kg to 48 μg/kg IC or 18 μg/kg to 36 μg/kg IV of rFGF-2 of SEQ ID NO: 2. When the 33 human CAD patients were assessed by resting cardiac magnetic resonance imaging (MRI) at baseline (*i.e.*, prior to treatment), and 1, 2 and 6 months after treatment with a single unit dose of rFGF-2 of the invention by IC or IV routes, the patients exhibited a highly statistically significant response to the method of treatment as objectively measured by increased target wall thickening, target wall motion, and target area collateral extent, and by decreased target area delayed arrival extent. (Table 7) By way of summary, at 1, 2 and 6 months, the target wall thickening increased relative to baseline at 4.4%, 6.3% and 7.7%, respectively; the target wall motion increased relative to baseline at 2.7%, 4.4% and 6.4%, respectively; the target area collateral extent increased relative to baseline at 8.3%, 10.9% and 11.2%, respectively; and the target area delayed

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a plot of the mean rFGF-2 plasma concentration versus time profiles for eight different doses of rFGF-2 (SEQ ID NO: 2) administered by IC infusion in humans over a 20 minute period. The eight doses of rFGF-2 presented in Figure 1A are 0.33, 0.65, 2, 6, 12, 24, 36, and 48 μg/kg of lean body mass (LBM).

Figure 1B is a plot of the mean FGF-2 plasma concentration versus time profiles for 2 different doses of rFGF-2 (SEQ ID NO: 2) administered by IV infusion in humans over a 20 minute period. The two IV doses of rFGF-2 in Figure 1B are 18 and 36 μg/kg. The mean concentration-time profile following IC administration of 36 μg/kg rFGF-2 is included for comparison.

Figure 2 is a plot of mean FGF-2 area under the curve (AUC) in pg*min/ml corresponding to Figures 1A and 1B. This plot shows the dose linearity of systemic rFGF-2 exposure following IC or IV infusion. The systemic exposure for the IC route is similar to that observed following IV administration.

Figure 3 is a plot of individual human patient FGF-2 plasma clearance (CL) values as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on rFGF-2 plasma clearance (CL).

Figure 4 is a plot individual human patient FGF-2 dose normalized area under curves (AUCs) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on FGF-2 AUC.

angiogenesis with an angiogenic effect of significant efficacy so as not to require retreatment for at least 4-6 months, typically 6 months. The unit dose composition of the present invention is typically provided in combination with one or more pharmaceutically acceptable excipients or carriers. In other embodiments of the unit dose composition, a safe and therapeutically effective amount comprises about 0.2 μg/kg to about 2 μg/kg, about 2 μg/kg to about 24 μg/kg, or about 24 μg/kg to about 48 μg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof.

It is convenient to define the unit dose composition of the present invention in more absolute terms that are not dependent upon the weight of the patient to be treated. When so defined, the unit dose composition comprises from .008 mg to 7.2 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. In this embodiment, the unit dose composition contains a sufficient amount of FGF-2 to accommodate dosing any one of the majority of human CAD patients, ranging from the smallest patient (*e.g.*, 40 kg) at the lowest dosage (about 0.2 μg/kg) through the larger patients (*e.g.*, 150 kg) at the highest dosage (about 48 μg/kg). More typically, the unit dose comprises 0.3 mg to 3.5 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. The unit dose composition is typically provided in solution or lyophilized form containing the above referenced amount of rFGF-2 and an effective amount of one or more pharmaceutically acceptable buffers, stabilizers and/or other excipients as later described herein.

The active agent in the Applicants' above described unit dose composition is a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. Methods for making recombinant FGF-2 are well-known in the art. The recombinant FGF-2 of SEQ ID NO: 2 is made as described in U.S. Patent 5,155,214, entitled "Basic Fibroblast Growth Factor," which issued on October 13, 1992, and which is expressly incorporated herein by reference in its entirety. Moreover, all other references cited herein, whether occurring before or after this sentence, are expressly incorporated herein by reference in their entirety. As disclosed in the '214 patent, a DNA of SEQ ID NO: 1, which encodes a bFGF (hereinafter "FGF-2") of SEQ ID NO: 2, is inserted into a cloning vector, such as pBR322, pMB9, Col E1, pCRI, RP4 or λ-phage, and the cloning vector is used to

and 1.95M NaCl ['455 at col. 9, lines 20-25]. Polypeptide homogeneity was confirmed by reverse-phase high pressure liquid chromatography (RP-HPLC). Buffer exchange was achieved by SEPHADEX® G-25(M) gel filtration chromatography.

In addition to the 146 residue rFGF-2 of SEQ ID NO: 2, the active agent in the unit dose of the present invention also comprises an "angiogenically active fragment" of FGF-2. By the term "angiogenically active fragment" of FGF-2 is meant a fragment of FGF-2 that has about 80% of the 146 residues of SEQ ID NO: 2 and that retains at least 50%, preferably at least 80%, of the angiogenic activity of the FGF-2 of SEQ ID NO: 2.

To be angiogenically active, the FGF-2 fragment should have two cell binding sites and at least one of the two heparin binding sites. The two putative cell binding sites of the analogous human FGF-2 occur at residue positions 36-39 and 77-81 thereof. See Yoshida, *et al.*, *"Genomic Sequence of hst, a Transforming Gene Encoding a Protein Homologous to Fibroblast Growth Factors and the int-2-Encoded Protein,"* PNAS USA, **84**:7305-7309 (Oct. 1987) at Fig. 3. The two putative heparin binding sites of hFGF-2 occur at residue positions 18-22 and 107-111 thereof. See Yoshida (1987) at Fig. 3. Given the greater than 98% similarity between the amino acid sequences for naturally occurring human FGF-2 (hFGF-2) and rFGF-2 (SEQ ID NO: 2), it is expected that the two cell binding sites for rFGF-2 (SEQ ID NO: 2) are also at residue positions 36-39 and 77-81 thereof, and that the two heparin binding sites are at residue positions 18-22 and 107-111 thereof. Consistent with the above, it is well known in the art that N-terminal truncations of the FGF-2 of SEQ ID NO: 2 do not eliminate its activity in cows. In particular, the art discloses several naturally occurring and biologically active fragments of the FGF-2 that have N-terminal truncations relative to the FGF-2 of SEQ ID NO: 2. An active and truncated bFGF-2 having residues 12-146 of SEQ ID NO: 2 was found in bovine liver and another active and truncated bFGF-2, having residues 16-146 of SEQ ID NO: 2 was found in the bovine kidney, adrenal glands and testes. [*See* U.S. Pat. 5,155,214 at col. 6, lines 41-46, citing to Ueno, *et al.*, Biochem and Biophys Res. Comm., **138**:580-588 (1986).] Likewise, other fragments of the

The phrase "sequence identity," as used herein, is intended to refer to the percentage of the same amino acids that are found similarly positioned within the mutein sequence when a specified, contiguous segment of the amino acid sequence of the mutein is aligned and compared to the amino acid sequence of the naturally occurring FGF-2.

When considering the percentage of amino acid sequence identity in the mutein, some amino acid residue positions may differ from the reference protein as a result of conservative amino acid substitutions, which do not affect the properties of the protein or protein function. In these instances, the percentage of sequence identity may be adjusted upwards to account for the similarity in conservatively substituted amino acids. Such adjustments are well-known in the art. *See, e.g.*, Meyers and Miller, *"Computer Applic. Bio. Sci.*, 4:11-17 (1988).

To prepare an "angiogenically active mutein" of an angiogenic agent of the present invention, one uses standard techniques for site directed mutagenesis, as known in the art and/or as taught in Gilman, *et al., Gene*, 8:81 (1979) or Roberts, *et al., Nature, 328*:731 (1987). Using one of the site directed mutagenesis techniques, one or more point mutations are introduced into the cDNA sequence of SEQ ID NO: 1 to introduce one or more amino acid substitutions or an internal deletion. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/hydrophilicity, and/or steric bulk of the amino acid being substituted. By way of example, substitutions between the following groups are conservative: Gly/Ala, Val/Ile/Leu, Lys/Arg, Asn/Gln, Glu/Asp, Ser/Cys/Thr, and Phe/Trp/Tyr. Significant (up to 35%) variation from the sequence of the naturally occurring angiogenic FGF-2 is permitted as long as the resulting protein or polypeptide retains angiogenic activity within the limits specified above.

Cysteine-depleted muteins are muteins within the scope of the present invention. These muteins are constructed using site directed mutagenesis as described above, or according to the method described in U.S. Pat. 4,959,314 ("the `314 patent"), entitled "Cysteine-Depleted Muteins of Biologically Active Proteins." The `314 patent discloses how to determine biological activity and the effect of the substitution. Cysteine substitution is particularly useful in proteins

or diluents for injectable or infusible solutions are nontoxic to a human recipient at the dosages and concentrations employed, and include sterile water, sugar solutions, saline solutions, protein solutions or combinations thereof.

Typically, the pharmaceutically acceptable carrier includes a buffer and one or more stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. The use of buffers, stabilizers, reducing agents, anti-oxidants and chelating agents in the preparation of protein based compositions, particularly pharmaceutical compositions, is well-known in the art. *See*, Wang *et al.*, *"Review of Excipients and pHs for Parenteral Products Used in the United States," J. Parent. Drug Assn.*, **34**(6):452-462 (1980); Wang *et al.*, *"Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," J. Parent. Sci. and Tech.*, **42**:S4-S26 (Supplement 1988); Lachman, *et al.*, *"Antioxidants and Chelating Agents as Stabilizers in Liquid Dosage Forms-Part 1," Drug and Cosmetic Industry*, **102**(1): 36-38, 40 and 146-148 (1968); Akers, M.J., *"Antioxidants in Pharmaceutical Products," J. Parent. Sci. and Tech.*, **36**(5):222-228 (1988); and Methods in Enzymology, Vol. XXV, Colowick and Kaplan Eds., *"Reduction of Disulfide Bonds in Proteins with Dithiothreitol,"* by Konigsberg, pages 185-188. Suitable buffers include acetate, adipate, benzoate, citrate, lactate, maleate, phosphate, tartarate and the salts of various amino acids. *See* Wang (1980) at page 455. Suitable stabilizers include carbohydrates such as threlose or glycerol. Suitable reducing agents, which maintain the reduction of reduced cysteines, include dithiothreitol (DTT also known as Cleland's reagent) or dithioerythritol at 0.01% to 0.1% wt/wt; acetylcysteine or cysteine at 0.1% to 0.5% (pH 2-3); and thioglycerol at 0.1% to 0.5% (pH 3.5 to 7.0) and glutathione. *See* Akers (1988) at pages 225 to 226. Suitable antioxidants include sodium bisulfite, sodium sulfite, sodium metabisulfite, sodium thiosulfate, sodium formaldehyde sulfoxylate, and ascorbic acid. *See* Akers (1988) at pages 225. Suitable chelating agents, which chelate trace metals to prevent the trace metal catalyzed oxidation of reduced cysteines, include citrate, tartarate, ethylenediaminetetraacetic acid (EDTA) in its disodium, tetrasodium, and calcium disodium salts, and diethylenetriamine pentaacetic acid (DTPA). *See e.g.*, Wang (1980) at pages 457-458 and 460-461, and Akers (1988) at

known volume or concentration with sterile aqueous diluent such as sterile water, a sterile sugar solution, or a sterile saline solution. Alternatively, it could be reconstituted with a sterile buffer solution as described above, but lacking a chelating agent, such as EDTA. As a lyophilized cake, the unit dose composition is stable from 6 months to two years at refrigerated temperatures. Thus, storage of the unit dose composition in lyophilized form is readily accommodated using conventional refrigeration equipment.

Because the unit dose composition of the present invention is administered via a cardiac catheter or other injection device, which has dead space, it is convenient to formulate the vial containing the unit dose composition so that it contains about 10-50% more of the rFGF-2 or angiogenically active fragment or mutein thereof than is to be administered to the patient. For example, when the unit dose of the rFGF-2 to be administered is 7.2 mg, the vial is optionally formulated to contain up to 50% extra (*e.g.*, a total of about 10.8 mg) of rFGF-2 or angiogenically active fragment or mutein thereof. The extra solution is suitable for filling the dead space in the delivery equipment. In an alternative embodiment that does not allow for dead space, the pharmaceutical composition is loaded in the cardiac catheter in front of a pharmaceutically acceptable buffer, diluent or carrier, which is then used to deliver the appropriate amount of the one or more dosages to the one or more sites in the myocardium that are in need of angiogenesis.

As discussed above, the pharmaceutically acceptable carrier for the above described unit dose composition comprises a buffer and one or more stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. It is also within the scope of the present invention that the unit dose composition contain an amount of a glycosoaminoglycan (also known as a "proteoglycan" or a "mucopolysaccharide"), such as heparin, that is effective to bind to the FGF-2 and to the endothelial cell receptors so as to enhance the angiogenic effectiveness of the FGF-2 or angiogenically active fragment or mutein thereof. The amount of heparin that is administered is about 10-80 U per kg of patient weight (U/kg), typically about 40 U/kg. Expressed in absolute terms, the total amount of heparin administered to any one patient does not exceed 5,000 U. Thus, upon

two) patent coronary vessels. When administered over a twenty minute period, the unit dose composition is typically administered at a rate of 0.5 to 2.0 ml/minute, more typically at about 1 ml/minute. The coronary vessels can be native vessels or grafts, so long as they are not occluded. The volume of the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is typically 10-40 ml; more typically 20 ml. The length of time for infusion of the unit dose is not critical and can be shortened or lengthened depending on the rate and volume of infusion

When administered as an intravenous (IV) infusion, the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is administered typically within an hour, more typically over a 20 minute period, into a peripheral vein using a conventional IV setup. When administered over a twenty minute period, the unit dose composition is typically administered at a rate of 1 ml/minute.

In the phase I clinical trial of the above described method for treating CAD, a single unit dose composition was administered IC or IV to human patients having CAD who remained symptomatic with angina despite optional medical management. Because the method of the present invention induces angiogenesis, the method of the present invention provides treatment of the underlying condition in CAD or MI and not merely transitory relief from the symptoms, such as provided by nitrates. Typically, the safe and therapeutically effective amount of the method of the present invention comprises 0.2 μg/kg to 48 μg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In other embodiments, the safe and therapeutically effective amount comprises 0.2 μg/kg to 2 μg/kg, >2 μg/kg to <24 μg/kg, or 24 μg/kg to 48 μg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In absolute terms, the safe and therapeutically effective amount is about .008 mg to about 7.2 mg of rFGF-2 or an angiogenically active fragment or mutein thereof; more typically, 0.3 mg to 3.5 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. A suitable rFGF-2 is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof.

In another aspect, the present invention is also directed to a method for inducing angiogenesis in a heart of a human patient comprising, administering a

criterion (ETT and/or SAQ) as early as 30 days following IC or IV administration of a single unit dose of the present invention, and was maintained for six months following dosing. In certain patients with progressive CAD disease, it may be necessary or appropriate to administer additional unit doses of rFGF-2 at six or 12 month intervals after the initial unit dose, to overcome the progression of the CAD during that interim period. In some patients with very progressive CAD, unit doses of present invention would be readministered at 4 month intervals. In any instance, the treating physician would be able to determine the time, if any, for readministration based upon routine assessment of the clinical symptoms of the patient.

One of the benefits of the method of the present invention is cardiac angiogenesis. Accordingly, in another aspect, the present invention is directed to a method for inducing angiogenesis in a heart of a human patient, comprising administering into one or more coronary vessels (IC) or into a peripheral vein (IV) of a human patient in need of coronary angiogenesis, a single unit dose composition comprising an angiogenically effective amount of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In the above method, the angiogenically effective amount comprises about 0.2 μg/kg to about 48 μg/kg (or in absolute terms about .008 mg to about 7.2 mg) of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. More typically, the angiogenically effective amount comprises about 0.3 mg to 3.5 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. A suitable rFGF-2 for use in the above-identified method is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment thereof. In one embodiment of the above method, the unit dose composition is administered IC to patent coronary vessels or IV to a peripheral vein. In another embodiment, the unit dose composition is administered with heparin as described herein.

The above described method for providing coronary angiogenesis is also beneficial in human patients that have undergone a myocardial infarction (MI) in one or more coronary arteries. Accordingly, in another aspect, the present invention is also directed to a method for treating a human patient for an MI

during and after rFGF-2 administration is also preferred. Finally, it is also within the scope of the above described methods to include the step of administering an effective amount of a glycosoaminoglycan (also known as a "proteoglycan" or a "mucopolysaccharide"), such as heparin from 0-30 minutes prior to administering the unit dose composition of the present invention. Typically, the effective amount of glycosaminoglycan (such as heparin) that is administered is about 10-80 U/kg, more typically, about 40 U/kg. However, the total amount of heparin administered to any one patient immediately prior to dosing generally does not exceed 5,000 U.

Because EDTA is a potent chelator of calcium which is required for normal myocardial contraction and cardiac conduction, minimizing the concentration of EDTA is critical to patient's safety. A concentration of EDTA less than 100 μg/ml optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

Because a sudden bolus of rFGF-2 is associated with profound hypotension in animals, the rate of infusion is critical to patient's safety. Administration at 0.5 to 2 mL per minute, typically 1 mL per minute, optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

A Phase I clinical trial directed to treating human patients for CAD by administering a single unit dose composition of the present invention was conducted and is described in Examples 1-3 herein. In that trial, sixty-six (66) human patients diagnosed with CAD, who satisfied the criteria of Example 2 herein, received a single unit dose of rFGF-2 in accordance with the method of the present invention. Specifically, fifty-two human patients were administered a unit dose of 0.33 μg/kg to 48 μg/kg of rFGF-2 by IC infusion over about a 20 minute period. Fourteen human patients were administered a unit dose of either 18 μg/kg or 36 μg/kg of rFGF-2 by IV infusion over about a 20 minute period. The 66 treated patients were then assessed relative to baseline (*i.e.*, prior to treatment with the single unit dose), and again at 1 month, 2 months and 6 months after treatment with the single unit dose, using three sets of art-recognized assessment criteria: 1) changes in their exercise tolerance time (ETT); 2) the Seattle Angina Questionnaire, which provides an assessment based upon a mixed combination of objective and

persistence of increased ETT at 6 months (133.1 sec and 87.5 sec) in the high dose IC (24-48 μg/kg) and IV (18 & 38 μg/kg) groups, respectively, was unexpected. The greatest mean increases in ETT of 107.9 and 133.1 seconds at 2 and 6 months, respectively, occurred in the high dose (24-48 μg/kg) IC group. The IV group exhibited significant mean increases in ETT of 93.4 seconds and 87.5 seconds, at 2 months and 6 months respectively, which was not predicted by the rat and pig animal models used herein. Overall, the persistence of the effect (increase in ETT) at six months and its magnitude for both the IC and IV groups was wholly unexpected.

The 66 human patients of the Phase I clinical trial described in Examples 1-3 herein were also evaluated using the Seattle Angina Questionnaire (SAQ). The SAQ is a validated, disease-specific, quality of life instrument which assesses the following five scales: 1) "exertional capacity" = limitation of physical activity; 2) "disease perception" = worry about MI; 3) "treatment satisfaction"; 4) "angina frequency" = number of episodes and sublingual nitroglycerin usage; and 5) "angina stability" = number of episodes with most strenuous physical activity. The possible range of scores for each of the five scales is 0 to 100 with the higher scores indicating a better quality of life. Typically, a mean change of 8 points or more between the mean baseline scores (*i.e.,* before treatment) and the post-treatment scores is recognized as being "clinically significant." However, in the present analysis, a dose was considered "effective" if the mean change in score from baseline increased by greater than 14 points. The reason that 14 was chosen (instead of 8) was to allow for the improvement that was seen in the placebo group at 2 months in a clinical trial of another growth factor--VEGF.

In performing the SAQ evaluation, the patients were categorized according to the same dosage groups that were evaluated for ETT, *i.e.*, 0.33 - 2.0 μg/kg IC (low) 6.0 - 12.0 μg/kg IC (mid); 24 - 48 μg/kg IC (high); and 18 and 36 μg/kg IV. The questionnaire was administered to subjects in each dosage group at baseline (prior to dosing), and at 2 months and 6 months after being administered a single unit dose composition of rFGF-2 in accordance with the method of the present invention.

According to Table 3, the change in score for angina stability increased relative to baseline at both 2 and 6 months for each group. The improvements in angina stability seen at 2 months after dosing (46.2, 32.1, 34.3 and 39.6) were significantly greater than those scores seen at 6 months (21.4, 16.7, 17.7 and 23.2). However, the scores found at both 2 months and 6 months after dosing showed that all dosages were found to be effective (>14) in increasing angina stability. Moreover, the magnitude of the increases and their duration for 6 months were unexpected.

The third SAQ scale to be evaluated was "angina frequency." The data summarizing the angina frequency is presented in Table 4 herein. **Table 4. Angina Frequency (AF) – Change from Baseline**

FGF-2 Dose Group	*Change from Baseline at Two Months*	*Change from Baseline at Six Months*
0.33 to 2.0 μg/kg IC (N = 16) (low)	N = 14 27.9* (-10 to 80)	N = 7 12.9 (-40 to 50)
6.0 and 12 μg/kg IC (N = 8) (mid)	N = 7 32.9* (0 to 80)	N = 6 36.7 (-10 to 90)
24.0 to 48.0 μg/kg IC (N = 28) (high)	N = 27 28.9* (-40 to 80)	N = 24 25.8* (-30 to 80)
18.0 and 36.0 μg/kg IV (N = 14)	N = 12 20.0* (0 to 90)	N = 14 11.4 (-30 to 60)
ALL GROUPS (N = 66)	N = 60 27.3	N = 51 21.4

N = number of subjects; mean (range); * = $p < 0.05$

According to Table 4, the mean patient scores (27.9, 32.9, 28.9 and 20.0) for angina frequency increased at 2 months (relative to baseline) by an effective amount (>14) for all dosage groups and for all modes of administration (IC or IV). The mean patient scores continued to increase at 6 months only for the mid dose (6.0 - 12.0 μg/kg) group, suggesting a peak effect at 2 months post-dosing. However, for the mid dose (6.0 - 12.0 μg/kg) and high dose (24.0 - 48.0 μg/kg) groups, the changes at 2 months and 6 months were similar, suggesting a persistent effect at 6 months on angina frequency. The third SAQ scale to be evaluated was "angina

Table 6. Disease Perception (DP) – Change from Baseline

Dose Group	*Change from Baseline at Two Months*	*Change from Baseline at Six Months*
0.33 to 2.0 μg/kg IC (N = 16) (low)	N = 14 29.2* (-8 to 58)	N = 7 26.2* (0 to 42)
6.0 and 12 μg/kg IC (N = 8) (mid)	N = 7 20.2* (-8 to 50)	N = 6 30.6* (0 to 58)
24.0 to 48.0 μg/kg IC (N = 28) (high)	N = 27 27.8* (-33 to 92)	N = 24 34.0* (-33 to 100)
18.0 and 36.0 μg/kg IV (N = 14)	N = 12 22.9* (-8 to 92)	N = 14 23.8* (-8 to 75)

N = number of subjects; mean (range); * = p < 0.05

Up to 60 of the human patients of the Phase I clinical trial described in Examples 1-3 herein were also evaluated using resting magnetic resonance imaging (MRI) scans of their heart. The resting MRI scans were performed on the patients at baseline, and at 1 month, 2 months and 6 months after dosing with a single unit dose composition of the present invention. The doses were considered "effective" based upon statistical significance ($p < 0.05$). The objective criteria assessed by the resting MRI scans are the following: (1) ejection fraction; (2) myocardial infarct extent (%); (3) normal wall thickening (4) normal wall motion (%); (5) target wall thickening (%); (6) target wall motion (%); (7) target wall area collateral extent (%); and (8) target area delayed arrival extent (%).

Based upon the resting MRI, no change in "ejection fraction" was observed at one month for any one group. The mean change from baseline for all groups (n = 33) at 1 month was an increase of 2.0% (p= 0.042). At two months, the mean change from baseline for the low dose IC group (n=13) was an increase of 8.1% (p=0.007); and for all groups (n=54), the mean change from baseline was an increase of 3.8% (p=0.001). At six months, the mean change from baseline for the high dose IC group (n=19) was 5.3% (p=0.023); for the IV group (n=3) was 11.1% (p=0.087); and for all groups (n=33) was 5.7% (p=0.001).

Thus, providing CAD patients with a single IC or IV infusion of rFGF-2 in accordance with the present invention provided the patients with a statistically significant physical improvement as objectively measured by MRI and other conventional criteria.

Pharmacokinetics and Metabolism

The molecular structure of FGF-2 contains a positively charged tail that is known to bind to proteoglycan chains (heparin and heparin-like structures) on cell surfaces and on the endothelial wall of the vasculature. *See* Moscatelli, *et al.*, *"Interaction of Basic Fibroblast Growth Factor with Extracellular Matrix and Receptors,"* Ann. NY Acad. Sci., **638**:177-181 (1981).

The kidneys and liver are the major organs for the elimination of rFGF-2. In particular, the kidneys have a protein cutoff of about 60 kD and thus retain serum albumin (MW 60 kD). However, FGF-2 (146 residues) has a molecular weight of about 16.5 kD. Accordingly, renal excretion is to be expected. In a radiolabelled biodistribution study of commercially available bovine FGF-2 (bFGF-2), both the liver and the kidney were shown to contain high counts of the radiolabelled bFGF-2 at 1 hour after IV or IC injection. In a published study, wherein another recombinant iodinated form of bFGF-2 was given to rats, the liver was identified as the major organ of elimination. Whalen *et al.*, *"The Fate of Intravenously Administered bFGF and the Effect of Heparin,"* Growth Factors, **1**:157-164 (1989). It is also known that FGF-2 binds in the general circulation to α_2-macroglobulin and that this complex is internalized by receptors on the Kupffer cells. Whalen *et al.* (1989) and LaMarre *et al.*, *"Cytokine Binding and Clearance Properties of Proteinase-Activated Alpha-2-Macroglobulins,"* Lab. Invest., **65**:3-14 (1991). Labelled FGF-2 fragments were not found in the plasma, but they were found in the urine and corresponded in size to intracellular breakdown products.

In preclinical testing, we determined the pharmacokinetics of rFGF-2 (SEQ ID NO: 2) after intravenous (IV) and intracoronary (IC) administration in domestic Yorkshire pigs, and after IV administration dosing in Sprague Dawley ("SD") rats. The pig models demonstrated linear pharmacokinetics (0.65 μg/kg -

Figure 3 is a plot of individual human patient plasma clearance (CL) values (ml/min/kg) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion." Figure 3 shows the influence of timing of heparin administration on FGF-2 plasma clearance (CL). Although Figure 3 shows that administering heparin up to 100 minutes prior to rFGF-2 decreases FGF-2 clearance, the preferred time for administering heparin is from 0-30 minutes prior the rFGF-2 administration, wherein the effect of the heparin on decreasing FGF-2 clearance is greatest.

Figure 4 is a plot individual human patient rFGF-2 dose normalized area under curves (AUCs) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on rFGF-2 AUC. Figure 4 shows that the greatest AUC/dose was achieved when an effective amount of a glycosoaminoglycan, such as heparin, was preadministered within 30 minutes or less of IC rFGF-2 infusion, more preferably within 20 minutes or less of IC or IV rFGF-2 infusion. Typically, an effective amount of a glycosoaminoglycan is 10-80 U/kg heparin.

The mean pharmacokinetic parameters for rFGF-2 in humans as a function of dosage and mode of administration are summarized in Table 8 herein. Referring to Table 8, the T½ for FGF-2 in humans was determined to range from 2.2 ± 3.7 hours at low dose (0.33-2.0 μg/kg) IC to 7.0 ± 3.5 hours at a dose of 18-36 μg/kg IV; given the limitations of the assay, the terminal half-life is estimated at 5-7 hours for all groups. The clearances of FGF-2 ranged from 13.2 to 18.2 L/hour/70kg man. Finally, the steady state volume (V_{ss}) was determined to range from 11.3 ± 10.4 L/70kg man to 16.8 ± 10.7 L/70kg man.

Table 8. Mean rFGF-2 PK Parameters in Humans

FGF-2 Dose μg/kg	*N*	*Route*	*CL (L/hr/70kg)*	*$t_{½}$ (h)*	*V_{ss} (L/70kg)*
0.3 - 2	16	IC	18.2±13.4	2.2± 3.7	11.3±10.4
6 - 12	8	IC	13.2± 7.3	3.1± 2.5	12.1± 4.9
24 - 48	28	IC	14.7± 8.3	6.3± 1.8	16.8 ±10.7
18 - 36	14	IV	13.9± 7.9	7.0± 3.5	16.4± 8.6

the total infusion volume was increased to a maximum of 40 ml when proportionately higher absolute amounts of FGF-2 were administered to patients with higher body weights.

EXAMPLE 2

"Selection Criteria For Patients With Coronary Artery Disease For Treatment With rFGF-2"

The following selection criteria were applied to Phase I patients with coronary artery disease, whose activities were limited by coronary ischemia despite optimal medical management, and who were not candidates for approved revascularization therapies:

Inclusion criteria: Subject is eligible if:

- Male or female, greater than or equal to 18 years of age
- Diagnosis of coronary artery disease (CAD)
- Suboptimal candidates for approved revascularization procedures, *e.g.*, angioplasty, stents, coronary artery bypass graft (CABG) (or refuses those interventions)
- Able to exercise at least three minutes using a modified Bruce protocol and limited by coronary ischemia
- Inducible and reversible defect of at least 20% myocardium on pharmacologically stressed thallium sestamibi scan
- CBC, platelets, serum chemistry within clinically acceptable range for required cardiac catheterization
- Normal INR, or if anticoagulated with Coumadin, INR <2.0
- Willing and able to give written informed consent to participate in this study, including all required study procedures and follow-up visits

Exclusion criteria: Subject is not eligible if:

- Malignancy: any history of malignancy within past ten years, with the exception of curatively treated basal cell carcinoma.

- Any condition which makes the subject unsuitable for participation in this study in the opinion of the investigator, *e.g.*, psychosis, severe mental retardation, inability to communicate with study personnel, drug or alcohol abuse.

EXAMPLE 3

"Phase I Clinical Study on Recombinant FGF-2 (SEQ ID NO: 2) Administered to Humans"

The Phase I CAD trial of this example is an open label, dose escalation study of recombinant fibroblast growth factor-2 (rFGF-2) for safety, tolerability and pharmacokinetics. The study was conducted at two sites: Beth Israel Deaconess Hospital (Harvard) in Boston, MA and Emory University Hospital in Atlanta, GA. Enrollment is complete. Subjects were treated with a single infusion of rFGF-2 on Day 1 and followed for 360 days; follow-up is not yet complete in some subjects.

The subject population consists of patients with advanced CAD who are exercise limited by coronary ischemia and are considered suboptimal candidates for (or do not want to undergo) one of the established revascularization procedures (*e.g.*, CABG, angioplasty -- with or without stent). The major exclusion criteria were history or suspicion of malignancy, uncompensated heart failure or left ventricular ejection fraction <20%, renal insufficiency or proteinuria, and various ocular conditions (e.g., proliferative diabetic retinopathy, severe non-proliferative retinopathy).

Sixty-six subjects have received rFGF-2 of SEQ ID NO: 2 in this trial: fifty-two received the rFGF-2 as an IC infusion and fourteen received it as an IV infusion. Each subject was observed in the hospital for at least twenty-four hours, and then followed as an outpatient for 360 days with follow-up visits (Days 15, 29, 57, 180 and 360). At least four subjects were studied at each dose; if no subject experienced dose-limiting toxicity as defined by the protocol within six days, the dose was escalated. The drug was administered as a single 20 minute infusion divided between two major sources of coronary blood supply (IC), using standard techniques for positioning a catheter into the patient's coronary artery (such as

Preliminary Results

The results presented here are unaudited and are based on a third interim analysis for sixty-six subjects with six months follow - up for all groups (1-10) and the serious adverse events (SAE) report of 29 July 1999 from Chiron Drug Safety. Data collection for the last visit (Day 360) and final analysis is in progress.

The starting dose of 0.33 μg/kg IC was escalated over eight sequential groups to 48 μg/kg IC, at which dose 2 of ten subjects experienced dose-limiting toxicity (hypotension) as defined by the protocol. Hypotension was manageable with fluids alone in all subjects (no vasopressors or assistive devices). At 36 μg/kg IC, only 1 of 10 subjects had dose-limiting toxicity which defined this dose as the maximally tolerated dose (MTD). Two additional groups were studied by IV infusion; four subjects of half the MTD (18 μg/kg) and ten subjects at the MTD (36 μg/kg).

Hypotension was dose-limiting in humans, as predicted by the animal model in Yorkshire pigs. However, 36.0 μg/kg rFGF-2 IC was tolerated in humans; whereas in pigs, 20.0 μg/kg rFGF-2 IC caused profound hypotension in one of two animals. Better tolerability in humans was attributed to aggressive fluid management and absence of general anesthesia.

As of 29 July 1999, thirty-three serious adverse events (SAEs) have occurred in 24/66 subjects, but were not dose-related. Fifteen (15) SAEs were considered at least possibly related to rFGF-2; whenever there was a difference between relatedness assigned by the investigator and the medical monitor, the more conservative relationship was assigned. SAE's were multiple in five subjects: 01103 (0.33 μg/kg IC), 01106 (0.65 μg/kg IC),. 01113 (2.0μg/kg IC), 01137 (36.0 μg/kg IV), 02101 (0.65 μg/kg IC).

The most frequent treatment-emergent adverse events (AEs) on Day 1 were transient systolic hypotension and transient bradycardia. The hypotension was dose-dependent and occurred more frequently at doses greater than or equal to (≥) 24 μg/kg IC; bradycardia was not dose-dependent. Other adverse events (AEs) which were deemed at least possibly related and appeared dose-related occurred within the first several days or week post infusion and included chest pain, shortness

The human patients in this study that were treated with a single IC or IV infusion of rFGF-2 of SEQ ID NO: 2 exhibited a mean increase in ETT of 1.5 to 2 minutes. *See* Table 1. This is especially significant because an increase in ETT of greater than (>) 30 seconds is considered significant and a benchmark for evaluating alternative therapies, such as angioplasty. The angina frequency and quality of life, as measured by SAQ, showed a significant improvement at 2 months in all five subscales for the 66 patients (n=66) tested. See Tables 26. In Tables 2-6, a mean change of 14 or more was considered "clinically significant."

When 33 human CAD patients were assessed by resting cardiac magnetic resonance imaging (MRI) at baseline, and at 1, 2, and 6 months after receiving a single unit dose composition of the present invention by IC or IV routes, a highly statistically significant increase was observed in target wall thickening, target wall motion and target area collateral extent; a highly statistically significant decrease was observed in target area delayed arrival extent; and no statistically significant changes were observed in normal wall motion, normal wall thickening or myocardial infarct extent.

In addition to the above criterion (*i.e.*, ETT SAQ, MRI), a treatment is considered very successful if the angiogenic effects last at least six months. In the present Phase I study, the unexpectedly superior angiogenic effects were observed to last up to 6 months in some patients in all dosage groups. Based upon the results already obtained, it is expected that the angiogenic effects may last twelve months or more but do last at least six months in the patients, at which time the procedure could be repeated, if necessary.

EXAMPLE 4

"Proposed Phase II Clinical Study On rFGF-2 (SEQ ID NO: 2) Administered to Humans to Treat Coronary Artery Disease"

The Phase II clinical trial of rFGF-2 for treating human patients for coronary artery disease is performed as a double blind/placebo controlled study having four arms: placebo, 0.3 μg/kg, 3.0 μg/kg, and 30 μg/kg administered once IC. This study is ongoing and results are not yet available.

CLAIMS

What is claimed is:

1. A unit dose composition for inducing angiogenesis in a human, comprising about .008 mg to about 7.2 mg of FGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier.

2 The unit dose composition of claim 1, comprising 0.3 mg to 3.5 mg of FGF-2, or an angiogenically active fragment or mutein thereof.

3. The unit dose composition of claim 1, wherein said FGF-2 has the amino acid sequence of SEQ ID NO: 2.

4. The unit dose composition of claim 3, comprising 0.3 mg to 3.5 mg of an FGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier.

5. The unit dose composition of claim 3, comprising about .008 mg to about 7.2 mg of said angiogenically active mutein of said FGF-2 of SEQ ID NO: 2 in a pharmaceutically acceptable carrier.

6. The unit dose composition of claim 5, comprising 0.3 mg to 3.5 mg of said angiogenically active mutein of said FGF-2 of SEQ ID NO: 2 in a pharmaceutically acceptable carrier.

13. The method of claim 12, wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof is administered to one or more coronary vessels.

14. The method of claim 13, wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof is about 24 μg/kg to 48 μg/kg.

15. The method of claim 12 wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or said angiogenically active fragment or mutein thereof is administered to a peripheral vein.

16. The method of claim 15, wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or said angiogenically active fragment or mutein thereof is about 18 μg/kg to 36 μg/kg.

17. A method for treating a human patient for coronary artery disease comprising, administering a single unit dose of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in a human patient in need of treatment for coronary artery disease, said unit dose comprising from about .008 mg to 7.2 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof.

25. The method of claim 19, further comprising the step of administering 10 U/kg to 80 U/kg of heparin to said patient IV or IC about 0 to 30 minutes prior to administering said unit dose.

26. A method for inducing angiogenesis in a heart of a human patient comprising, administering a single unit dose of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in a human patient in need of treatment for coronary artery disease, said unit dose comprising from about .008 mg to 7.2 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof.

27. The method of claim 26, wherein said FGF-2 has the amino acid sequence of SEQ ID NO: 2.

28. The method of claim 27 wherein said single unit dose produces an improvement in one or more clinical endpoints in said human patient that lasts at least four months.

29. The method of claim 28, wherein said single unit dose produces an improvement in one or more clinical endpoints in said human patient that lasts 6 months.

30. A method for treating a human patient for a myocardial infarction comprising, administering a single unit dose of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in said human patient, said unit dose comprising from about

Fig. 1A

Mean rFGF Plasma Concentration Versus Time Post IC Administration

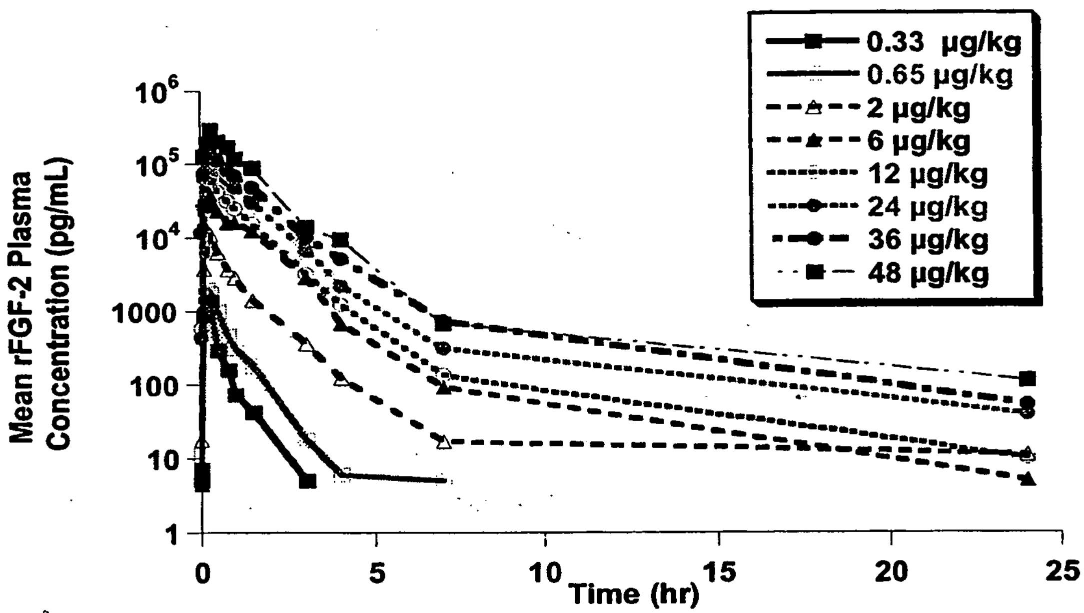


Fig. 2

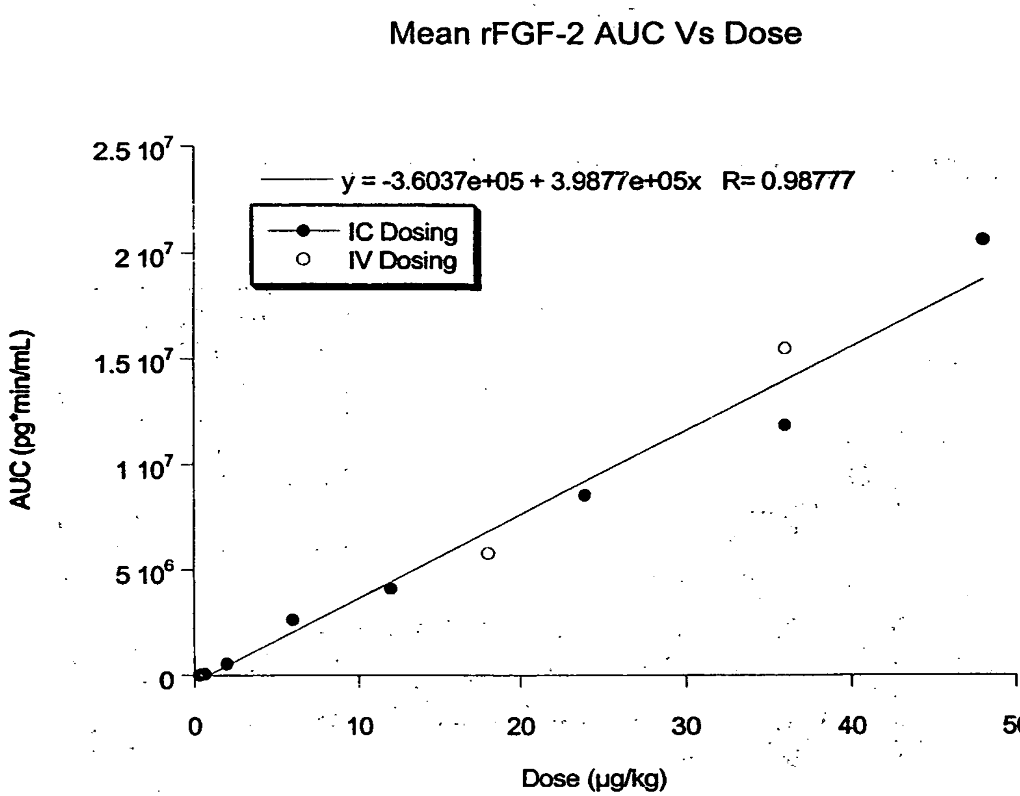
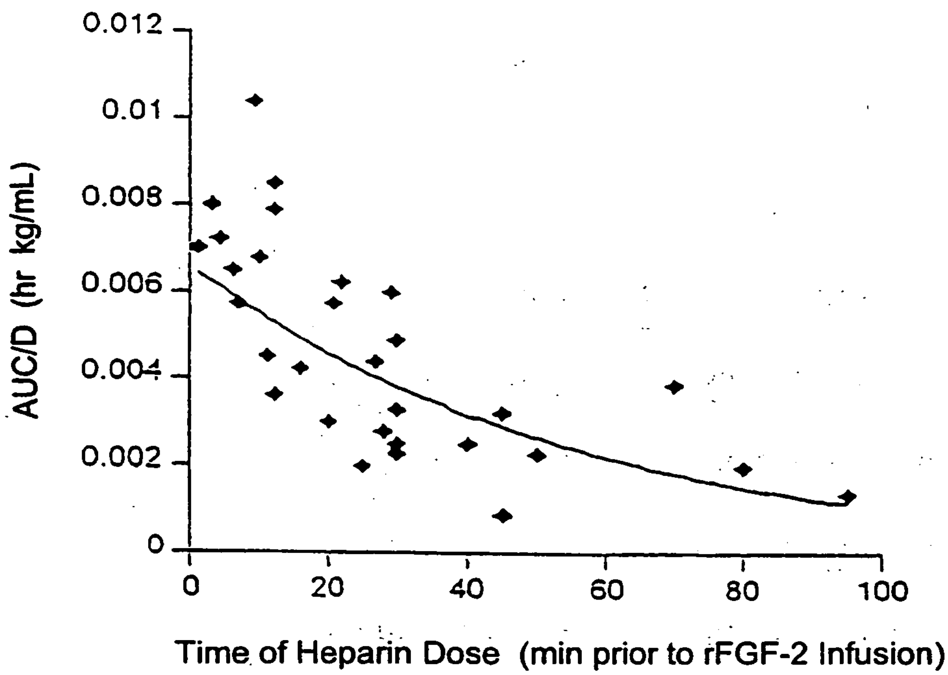

Mean rFGF-2 AUC Vs Dose
y = -3.6037e+05 + 3.9877e+05x R= 0.98777
IC Dosing
IV Dosing
AUC (pg*min/mL)
2.5 10^7
2 10^7
1.5 10^7
1 10^7
5 10^6
0
0
10
20
30
40
50
Dose (µg/kg)

Fig. 4

Individual Patient rFGF-2 Dose-Normalized AUC Versus Dose in Study CS-FG001



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100 105 110

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Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Pro
115 120 125

aaa acc ggg cca ggg caa aaa gcc atc cta ttc cta cca atg tcc gcc 432
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aaa tcc taag 442
Lys Ser
145

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<212> PRT
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35 40 45

Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser
50 55 60

Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly
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100 105 110

Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Pro
115 120 125

Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala
130 135 140

Lys Ser
145

PCT

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A3

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With international search report.

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(54) Title: ANGIOGENICALLY EFFECTIVE UNIT DOSE OF FGF–2 AND METHOD OF USE

(57) Abstract

The present invention has multiple aspects. In particular, in one aspect, the present invention is directed to a unit dose composition comprising 0.2 μg/kg to 48 μg/kg of an FGF–2 of SEQ ID NO: 2, or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In another aspect, the present invention is directed to a method for treating a human patient for coronary artery disease, comprising administering into one or more coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease a safe and angiogenically effective dose of a recombinant FGF–2, or an angiogenically active fragment or mutein thereof. The single unit dose composition of the present invention provides an angiogenic effect in a human CAD patient that lasts six months before re–treatment is required. In another aspect, the present invention is directed to a method of administration which optimizes patient's safety. In this embodiment, fluids, heparin and/or rate of infusion all play a role. In another aspect, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of FGF–2, alone or in combination with heparin, in a therapeutically effective carrier. The magnitude and duration of benefit were unexpected; in addition benefit with the IV route was unexpected.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/19770

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/18 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SELLKE FRANK W ET AL: "Therapeutic angiogenesis with basic fibroblast growth factor: Technique and early results." ANNALS OF THORACIC SURGERY JUNE, 1998, vol. 65, no. 6, June 1998 (1998-06), pages 1540-1544, XP000891950 ISSN: 0003-4975 the whole document --- -/--	1,9-34

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2000	03/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/19770

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

CORRECTED VERSION*

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International Bureau

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(57) Abstract

The present invention has multiple aspects. In particular, in one aspect, the present invention is directed to a unit dose composition comprising 0.2 μg/kg to 48 μg/kg of an FGF–2 of SEQ ID NO: 2, or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In another aspect, the present invention is directed to a method for treating a human patient for coronary artery disease, comprising administering into one or more coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease a safe and angiogenically effective dose of a recombinant FGF–2, or an angiogenically active fragment or mutein thereof. The single unit dose composition of the present invention provides an angiogenic effect in a human CAD patient that lasts six months before re–treatment is required. In another aspect, the present invention is directed to a method of administration which optimizes patient's safety. In this embodiment, fluids, heparin and/or rate of infusion all play a role. In another aspect, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of FGF–2, alone or in combination with heparin, in a therapeutically effective carrier. The magnitude and duration of benefit were unexpected; in addition benefit with the IV route was unexpected.

*(Referred to in PCT Gazette No. 40/2000, Section II)

ANGIOGENICALLY EFFECTIVE UNIT DOSE OF FGF-2 AND METHOD OF USE

BACKGROUND OF THE INVENTION

A. Field of the Invention

The present invention is directed to a unit dose composition for inducing cardiac angiogenesis in a human comprising an therapeutically effective amount FGF-2 or an angiogenically active fragment or mutein thereof. The present invention is also directed to a method for administering a single unit dose composition to a human to induce cardiac angiogenesis while minimizing systemic risk to the patient. The present invention is useful because the disclosed unit dose composition, and method for its administration, provide an alternative to angioplasty or surgical intervention for the treatment of coronary artery disease (CAD) and further provide an adjunct for reducing post myocardial infarct (MI) injury in humans.

B. Background of the Invention

The fibroblast growth factors (FGF) are a family of at least eighteen structurally related polypeptides (named FGF-1 to FGF-18) that are characterized by a high degree of affinity for proteoglycans, such as heparin. The various FGF molecules range in size from 15-23 kD, and exhibit a broad range of biological activities in normal and malignant conditions including nerve cell adhesion and differentiation [Schubert *et al., J. Cell Biol. **104:***635-643 (1987)]; wound healing [U.S. Patent 5,439,818 (Fiddes)]; as mitogens toward many mesodermal and ectodermal cell types, as trophic factors, as differentiation inducing or inhibiting factors [Clements, *et al.*, *Oncogene 8:*1311-1316 (1993)]; and as an angiogenic factor [Harada, *J. Clin. Invest., 94:*623-630 (1994)]. Thus, the FGF family is a family of pluripotent growth factors that stimulate to varying extents fibroblasts, smooth muscle cells, epithelial cells and neuronal cells.

Many of the various FGF molecules have been isolated and administered to various animal models of myocardial ischemia with varying and often times opposite results. According to Battler *et al.*, "the canine model of myocardial ischemia has been criticized because of the abundance of naturally occurring collateral circulation, as opposed to the porcine model, which 'excels' in its relative paucity of natural collateral circulation and its resemblance to the human coronary circulation." Battler *et al.*, *"Intracoronary Injection of Basic Fibroblast Growth Factor Enhances Angiogenesis in Infarcted Swine Myocardium," JACC, 22(7)*: 2001-6 (Dec. 1993) at page 2002, col.1. However, Battler *et al.*, who administered bovine bFGF (*i.e.*, FGF-2) to pigs in a myocardial infarct model, considered the varying results that are obtained from one animal species to another, and expressly discloses that the divergent results "thus emphasiz[e] the caution that must be exercised in extrapolating results from different animal models." Battler *et al.*, at page 2005, col.1. Further, Battler points out that "the dosage and mode of administration of bFGF [i.e., bovine FGF-2] may have profound implications for the biologic effect achieved." Battler, *et al.*, at page 2005, col.1. Thus, it is a further object of this invention to discover a dosage and a mode of administration of a fibroblast growth factor that would provide for the safe and efficacious treatment of CAD and/or post MI injury in a human patient. More generally, it is an object of the present invention to provide a pharmaceutical composition and method for inducing angiogenesis in a human heart.

In another aspect, the present invention is directed to a method of treating a human patient for CAD or to induce coronary angiogenesis therein. The method comprises administering into one or more coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease (or in need of angiogenesis) a safe and therapeutically effective amount of a recombinant FGF-2 (rFGF-2) or an angiogenically active fragment or mutein thereof. Typically, a portion of the safe and therapeutically effective amount is administered to each of two coronary vessels. The safe and therapeutically effective amount comprises about 0.2 μg/kg to about 48 μg/kg, of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In other embodiments, the safe and therapeutically effective amount comprises 0.2 μg/kg to 2 μg/kg, >2 μg/kg to <24 μg/kg, or 24 μg/kg to 48 μg/kg of rFGF-2 an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In absolute terms, the amount of rFGF-2 or angiogenically active fragment or mutein thereof that is used in the above method comprises .008 mg to 7.2 mg, more typically 0.3 mg to 3.5 mg, of rFGF-2 or an angiogenically active fragment or mutein thereof.

Because FGF-2 is a glycosoaminoglycan (*e.g.*, heparin) binding protein and the presence of a glycosoaminoglycan optimizes activity and AUC (see Figs. 3 and 4), the IC dosages of RFGF-2 of the present invention typically are administered from 0-30 minutes prior to the administration of a glycosoaminoglycan, such as a heparin. The heparin is administered IC or IV, typically IV. Optionally, the heparin is combined with the unit dose composition.

Because rFGF-2 releases nitric oxide which is a potent vasodilator, aggressive fluid management prior to (proactively) and during the infusion is critical to patient's safety. Administration of IV fluids (e.g., 500-1000 mL of normal saline) to establish a wedge pressure of 12 mm Hg prior to infusion and administration of boluses of IV fluids (e.g., 200 mL normal saline) for decreases of systolic blood pressure (e.g., <90 mm Hg) associated with infusion optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

Because EDTA is a potent chelator of calcium which is required for normal myocardial contraction and cardiac conduction, minimizing the

significant manner for all dosage ranges whether administered IC or IV. (Tables 2-6). In particular, the five scales assessed by the SAQ are exertional capacity, angina stability, angina frequency, treatment satisfaction, and disease perception. Relative to the baseline, the mean score for exertional capacity increased by 10.9 to 20.2 at 2 months; and by 16.5 to 24.1 at 6 months. For angina stability, the mean score increased by 32.1 to 46.2 at 2 months; and by 16.7 to 23.2 at 6 months. For angina frequency, the mean score increased by 20.0 to 32.9 at 2 months; and by 11.4 to 36.7 at 6 months. For treatment satisfaction, the mean score increased by 8.5 to 19.8 at 2 months; and by 6.3 to 19.8 at 6 months. For disease perception, the mean score increased by 20.2 to 27.8 at 2 months; and by 23.8 to 34.0 at 6 months. Generally, a change of 8 points on any scale is considered clinically significant. Thus, the observed changes of 8.5-46.2 are clinically significant for each of the five scales that were assessed. Even assuming a placebo effect whereby a mean change from baseline of 14 points is considered clinically significant, the results still provide for an unexpectedly superior effect at almost all scales that were assessed.

As part of this study, MRI was also performed on 33 human patients diagnosed with CAD to assess the effect of administering a single unit dose of rFGF-2 on their cardiac ejection fraction, regional myocardial function and perfusion (delayed arrival zone). Specifically, the patients were administered a single unit dose of 0.33 μg/kg to 48 μg/kg IC or 18 μg/kg to 36 μg/kg IV of rFGF-2 of SEQ ID NO: 2. When the 33 human CAD patients were assessed by resting cardiac magnetic resonance imaging (MRI) at baseline (*i.e.*, prior to treatment), and 1, 2 and 6 months after treatment with a single unit dose of rFGF-2 of the invention by IC or IV routes, the patients exhibited a highly statistically significant response to the method of treatment as objectively measured by increased target wall thickening, target wall motion, and target area collateral extent, and by decreased target area delayed arrival extent. (Table 7) By way of summary, at 1, 2 and 6 months, the target wall thickening increased relative to baseline at 4.4%, 6.3% and 7.7%, respectively; the target wall motion increased relative to baseline at 2.7%, 4.4% and 6.4%, respectively; the target area collateral extent increased relative to baseline at 8.3%, 10.9% and 11.2%, respectively; and the target area delayed

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a plot of the mean rFGF-2 plasma concentration versus time profiles for eight different doses of rFGF-2 (SEQ ID NO: 2) administered by IC infusion in humans over a 20 minute period. The eight doses of rFGF-2 presented in Figure 1A are 0.33, 0.65, 2, 6, 12, 24, 36, and 48 μg/kg of lean body mass (LBM).

Figure 1B is a plot of the mean FGF-2 plasma concentration versus time profiles for 2 different doses of rFGF-2 (SEQ ID NO: 2) administered by IV infusion in humans over a 20 minute period. The two IV doses of rFGF-2 in Figure 1B are 18 and 36 μg/kg. The mean concentration-time profile following IC administration of 36 μg/kg rFGF-2 is included for comparison.

Figure 2 is a plot of mean FGF-2 area under the curve (AUC) in pg*min/ml corresponding to Figures 1A and 1B. This plot shows the dose linearity of systemic rFGF-2 exposure following IC or IV infusion. The systemic exposure for the IC route is similar to that observed following IV administration.

Figure 3 is a plot of individual human patient FGF-2 plasma clearance (CL) values as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on rFGF-2 plasma clearance (CL).

Figure 4 is a plot individual human patient FGF-2 dose normalized area under curves (AUCs) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on FGF-2 AUC.

angiogenesis with an angiogenic effect of significant efficacy so as not to require retreatment for at least 4-6 months, typically 6 months. The unit dose composition of the present invention is typically provided in combination with one or more pharmaceutically acceptable excipients or carriers. In other embodiments of the unit dose composition, a safe and therapeutically effective amount comprises about 0.2 μg/kg to about 2 μg/kg, about 2 μg/kg to about 24 μg/kg, or about 24 μg/kg to about 48 μg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof.

It is convenient to define the unit dose composition of the present invention in more absolute terms that are not dependent upon the weight of the patient to be treated. When so defined, the unit dose composition comprises from .008 mg to 7.2 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. In this embodiment, the unit dose composition contains a sufficient amount of FGF-2 to accommodate dosing any one of the majority of human CAD patients, ranging from the smallest patient (*e.g.*, 40 kg) at the lowest dosage (about 0.2 μg/kg) through the larger patients (*e.g.*, 150 kg) at the highest dosage (about 48 μg/kg). More typically, the unit dose comprises 0.3 mg to 3.5 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. The unit dose composition is typically provided in solution or lyophilized form containing the above referenced amount of rFGF-2 and an effective amount of one or more pharmaceutically acceptable buffers, stabilizers and/or other excipients as later described herein.

The active agent in the Applicants' above described unit dose composition is a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. Methods for making recombinant FGF-2 are well-known in the art. The recombinant FGF-2 of SEQ ID NO: 2 is made as described in U.S. Patent 5,155,214, entitled "Basic Fibroblast Growth Factor," which issued on October 13, 1992, and which is expressly incorporated herein by reference in its entirety. Moreover, all other references cited herein, whether occurring before or after this sentence, are expressly incorporated herein by reference in their entirety. As disclosed in the '214 patent, a DNA of SEQ ID NO: 1, which encodes a bFGF (hereinafter "FGF-2") of SEQ ID NO: 2, is inserted into a cloning vector, such as pBR322, pMB9, Col E1, pCRI, RP4 or λ-phage, and the cloning vector is used to

and 1.95M NaCl ['455 at col. 9, lines 20-25]. Polypeptide homogeneity was confirmed by reverse-phase high pressure liquid chromatography (RP-HPLC). Buffer exchange was achieved by SEPHADEX® G-25(M) gel filtration chromatography.

In addition to the 146 residue rFGF-2 of SEQ ID NO: 2, the active agent in the unit dose of the present invention also comprises an "angiogenically active fragment" of FGF-2. By the term "angiogenically active fragment" of FGF-2 is meant a fragment of FGF-2 that has about 80% of the 146 residues of SEQ ID NO: 2 and that retains at least 50%, preferably at least 80%, of the angiogenic activity of the FGF-2 of SEQ ID NO: 2.

To be angiogenically active, the FGF-2 fragment should have two cell binding sites and at least one of the two heparin binding sites. The two putative cell binding sites of the analogous human FGF-2 occur at residue positions 36-39 and 77-81 thereof. See Yoshida, *et al.*, *"Genomic Sequence of hst, a Transforming Gene Encoding a Protein Homologous to Fibroblast Growth Factors and the int-2-Encoded Protein,"* PNAS USA, 84:7305-7309 (Oct. 1987) at Fig. 3. The two putative heparin binding sites of hFGF-2 occur at residue positions 18-22 and 107-111 thereof. See Yoshida (1987) at Fig. 3. Given the greater than 98% similarity between the amino acid sequences for naturally occurring human FGF-2 (hFGF-2) and rFGF-2 (SEQ ID NO: 2), it is expected that the two cell binding sites for rFGF-2 (SEQ ID NO: 2) are also at residue positions 36-39 and 77-81 thereof, and that the two heparin binding sites are at residue positions 18-22 and 107-111 thereof. Consistent with the above, it is well known in the art that N-terminal truncations of the FGF-2 of SEQ ID NO: 2 do not eliminate its activity in cows. In particular, the art discloses several naturally occurring and biologically active fragments of the FGF-2 that have N-terminal truncations relative to the FGF-2 of SEQ ID NO: 2. An active and truncated bFGF-2 having residues 12-146 of SEQ ID NO: 2 was found in bovine liver and another active and truncated bFGF-2, having residues 16-146 of SEQ ID NO: 2 was found in the bovine kidney, adrenal glands and testes. [*See* U.S. Pat. 5,155,214 at col. 6, lines 41-46, citing to Ueno, *et al.*, Biochem and Biophys Res. Comm., 138:580-588 (1986).] Likewise, other fragments of the

The phrase "sequence identity," as used herein, is intended to refer to the percentage of the same amino acids that are found similarly positioned within the mutein sequence when a specified, contiguous segment of the amino acid sequence of the mutein is aligned and compared to the amino acid sequence of the naturally occurring FGF-2.

When considering the percentage of amino acid sequence identity in the mutein, some amino acid residue positions may differ from the reference protein as a result of conservative amino acid substitutions, which do not affect the properties of the protein or protein function. In these instances, the percentage of sequence identity may be adjusted upwards to account for the similarity in conservatively substituted amino acids. Such adjustments are well-known in the art. *See, e.g.,* Meyers and Miller, *"Computer Applic. Bio. Sci.*, 4:11-17 (1988).

To prepare an "angiogenically active mutein" of an angiogenic agent of the present invention, one uses standard techniques for site directed mutagenesis, as known in the art and/or as taught in Gilman, *et al., Gene*, 8:81 (1979) or Roberts, *et al., Nature, 328*:731 (1987). Using one of the site directed mutagenesis techniques, one or more point mutations are introduced into the cDNA sequence of SEQ ID NO: 1 to introduce one or more amino acid substitutions or an internal deletion. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/hydrophilicity, and/or steric bulk of the amino acid being substituted. By way of example, substitutions between the following groups are conservative: Gly/Ala, Val/Ile/Leu, Lys/Arg, Asn/Gln, Glu/Asp, Ser/Cys/Thr, and Phe/Trp/Tyr. Significant (up to 35%) variation from the sequence of the naturally occurring angiogenic FGF-2 is permitted as long as the resulting protein or polypeptide retains angiogenic activity within the limits specified above.

Cysteine-depleted muteins are muteins within the scope of the present invention. These muteins are constructed using site directed mutagenesis as described above, or according to the method described in U.S. Pat. 4,959,314 ("the `314 patent"), entitled "Cysteine-Depleted Muteins of Biologically Active Proteins." The `314 patent discloses how to determine biological activity and the effect of the substitution. Cysteine substitution is particularly useful in proteins

or diluents for injectable or infusible solutions are nontoxic to a human recipient at the dosages and concentrations employed, and include sterile water, sugar solutions, saline solutions, protein solutions or combinations thereof.

Typically, the pharmaceutically acceptable carrier includes a buffer and one or more stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. The use of buffers, stabilizers, reducing agents, anti-oxidants and chelating agents in the preparation of protein based compositions, particularly pharmaceutical compositions, is well-known in the art. *See*, Wang *et al.*, *"Review of Excipients and pHs for Parenteral Products Used in the United States," J. Parent. Drug Assn.*, 34(6):452-462 (1980); Wang *et al.*, *"Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," J. Parent. Sci. and Tech.*, **42**:S4-S26 (Supplement 1988); Lachman, *et al.*, *"Antioxidants and Chelating Agents as Stabilizers in Liquid Dosage Forms-Part 1," Drug and Cosmetic Industry*, **102**(1): 36-38, 40 and 146-148 (1968); Akers, M.J., *"Antioxidants in Pharmaceutical Products," J. Parent. Sci. and Tech.*, **36**(5):222-228 (1988); and Methods in Enzymology, Vol. XXV, Colowick and Kaplan Eds., *"Reduction of Disulfide Bonds in Proteins with Dithiothreitol,"* by Konigsberg, pages 185-188. Suitable buffers include acetate, adipate, benzoate, citrate, lactate, maleate, phosphate, tartarate and the salts of various amino acids. *See* Wang (1980) at page 455. Suitable stabilizers include carbohydrates such as threlose or glycerol. Suitable reducing agents, which maintain the reduction of reduced cysteines, include dithiothreitol (DTT also known as Cleland's reagent) or dithioerythritol at 0.01% to 0.1% wt/wt; acetylcysteine or cysteine at 0.1% to 0.5% (pH 2-3); and thioglycerol at 0.1% to 0.5% (pH 3.5 to 7.0) and glutathione. *See* Akers (1988) at pages 225 to 226. Suitable antioxidants include sodium bisulfite, sodium sulfite, sodium metabisulfite, sodium thiosulfate, sodium formaldehyde sulfoxylate, and ascorbic acid. *See* Akers (1988) at pages 225. Suitable chelating agents, which chelate trace metals to prevent the trace metal catalyzed oxidation of reduced cysteines, include citrate, tartarate, ethylenediaminetetraacetic acid (EDTA) in its disodium, tetrasodium, and calcium disodium salts, and diethylenetriamine pentaacetic acid (DTPA). *See e.g.*, Wang (1980) at pages 457-458 and 460-461, and Akers (1988) at

known volume or concentration with sterile aqueous diluent such as sterile water, a sterile sugar solution, or a sterile saline solution. Alternatively, it could be reconstituted with a sterile buffer solution as described above, but lacking a chelating agent, such as EDTA. As a lyophilized cake, the unit dose composition is stable from 6 months to two years at refrigerated temperatures. Thus, storage of the unit dose composition in lyophilized form is readily accommodated using conventional refrigeration equipment.

Because the unit dose composition of the present invention is administered via a cardiac catheter or other injection device, which has dead space, it is convenient to formulate the vial containing the unit dose composition so that it contains about 10-50% more of the rFGF-2 or angiogenically active fragment or mutein thereof than is to be administered to the patient. For example, when the unit dose of the rFGF-2 to be administered is 7.2 mg, the vial is optionally formulated to contain up to 50% extra (*e.g.*, a total of about 10.8 mg) of rFGF-2 or angiogenically active fragment or mutein thereof. The extra solution is suitable for filling the dead space in the delivery equipment. In an alternative embodiment that does not allow for dead space, the pharmaceutical composition is loaded in the cardiac catheter in front of a pharmaceutically acceptable buffer, diluent or carrier, which is then used to deliver the appropriate amount of the one or more dosages to the one or more sites in the myocardium that are in need of angiogenesis.

As discussed above, the pharmaceutically acceptable carrier for the above described unit dose composition comprises a buffer and one or more stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. It is also within the scope of the present invention that the unit dose composition contain an amount of a glycosoaminoglycan (also known as a "proteoglycan" or a "mucopolysaccharide"), such as heparin, that is effective to bind to the FGF-2 and to the endothelial cell receptors so as to enhance the angiogenic effectiveness of the FGF-2 or angiogenically active fragment or mutein thereof. The amount of heparin that is administered is about 10-80 U per kg of patient weight (U/kg), typically about 40 U/kg. Expressed in absolute terms, the total amount of heparin administered to any one patient does not exceed 5,000 U. Thus, upon

two) patent coronary vessels. When administered over a twenty minute period, the unit dose composition is typically administered at a rate of 0.5 to 2.0 ml/minute, more typically at about 1 ml/minute. The coronary vessels can be native vessels or grafts, so long as they are not occluded. The volume of the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is typically 10-40 ml; more typically 20 ml. The length of time for infusion of the unit dose is not critical and can be shortened or lengthened depending on the rate and volume of infusion

When administered as an intravenous (IV) infusion, the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is administered typically within an hour, more typically over a 20 minute period, into a peripheral vein using a conventional IV setup. When administered over a twenty minute period, the unit dose composition is typically administered at a rate of 1 ml/minute.

In the phase I clinical trial of the above described method for treating CAD, a single unit dose composition was administered IC or IV to human patients having CAD who remained symptomatic with angina despite optional medical management. Because the method of the present invention induces angiogenesis, the method of the present invention provides treatment of the underlying condition in CAD or MI and not merely transitory relief from the symptoms, such as provided by nitrates. Typically, the safe and therapeutically effective amount of the method of the present invention comprises 0.2 μg/kg to 48 μg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In other embodiments, the safe and therapeutically effective amount comprises 0.2 μg/kg to 2 μg/kg, >2 μg/kg to <24 μg/kg, or 24 μg/kg to 48 μg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In absolute terms, the safe and therapeutically effective amount is about .008 mg to about 7.2 mg of rFGF-2 or an angiogenically active fragment or mutein thereof; more typically, 0.3 mg to 3.5 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. A suitable rFGF-2 is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof.

In another aspect, the present invention is also directed to a method for inducing angiogenesis in a heart of a human patient comprising, administering a

criterion (ETT and/or SAQ) as early as 30 days following IC or IV administration of a single unit dose of the present invention, and was maintained for six months following dosing. In certain patients with progressive CAD disease, it may be necessary or appropriate to administer additional unit doses of rFGF-2 at six or 12 month intervals after the initial unit dose, to overcome the progression of the CAD during that interim period. In some patients with very progressive CAD, unit doses of present invention would be readministered at 4 month intervals. In any instance, the treating physician would be able to determine the time, if any, for readministration based upon routine assessment of the clinical symptoms of the patient.

One of the benefits of the method of the present invention is cardiac angiogenesis. Accordingly, in another aspect, the present invention is directed to a method for inducing angiogenesis in a heart of a human patient, comprising administering into one or more coronary vessels (IC) or into a peripheral vein (IV) of a human patient in need of coronary angiogenesis, a single unit dose composition comprising an angiogenically effective amount of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In the above method, the angiogenically effective amount comprises about 0.2 µg/kg to about 48 µg/kg (or in absolute terms about .008 mg to about 7.2 mg) of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. More typically, the angiogenically effective amount comprises about 0.3 mg to 3.5 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. A suitable rFGF-2 for use in the above-identified method is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment thereof. In one embodiment of the above method, the unit dose composition is administered IC to patent coronary vessels or IV to a peripheral vein. In another embodiment, the unit dose composition is administered with heparin as described herein.

The above described method for providing coronary angiogenesis is also beneficial in human patients that have undergone a myocardial infarction (MI) in one or more coronary arteries. Accordingly, in another aspect, the present invention is also directed to a method for treating a human patient for an MI

during and after rFGF-2 administration is also preferred. Finally, it is also within the scope of the above described methods to include the step of administering an effective amount of a glycosoaminoglycan (also known as a "proteoglycan" or a "mucopolysaccharide"), such as heparin from 0-30 minutes prior to administering the unit dose composition of the present invention. Typically, the effective amount of glycosaminoglycan (such as heparin) that is administered is about 10-80 U/kg, more typically, about 40 U/kg. However, the total amount of heparin administered to any one patient immediately prior to dosing generally does not exceed 5,000 U.

Because EDTA is a potent chelator of calcium which is required for normal myocardial contraction and cardiac conduction, minimizing the concentration of EDTA is critical to patient's safety. A concentration of EDTA less than 100 μg/ml optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

Because a sudden bolus of rFGF-2 is associated with profound hypotension in animals, the rate of infusion is critical to patient's safety. Administration at 0.5 to 2 mL per minute, typically 1 mL per minute, optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

A Phase I clinical trial directed to treating human patients for CAD by administering a single unit dose composition of the present invention was conducted and is described in Examples 1-3 herein. In that trial, sixty-six (66) human patients diagnosed with CAD, who satisfied the criteria of Example 2 herein, received a single unit dose of rFGF-2 in accordance with the method of the present invention. Specifically, fifty-two human patients were administered a unit dose of 0.33 μg/kg to 48 μg/kg of rFGF-2 by IC infusion over about a 20 minute period. Fourteen human patients were administered a unit dose of either 18 μg/kg or 36 μg/kg of rFGF-2 by IV infusion over about a 20 minute period. The 66 treated patients were then assessed relative to baseline (*i.e.*, prior to treatment with the single unit dose), and again at 1 month, 2 months and 6 months after treatment with the single unit dose, using three sets of art-recognized assessment criteria: 1) changes in their exercise tolerance time (ETT); 2) the Seattle Angina Questionnaire, which provides an assessment based upon a mixed combination of objective and

persistence of increased ETT at 6 months (133.1 sec and 87.5 sec) in the high dose IC (24-48 μg/kg) and IV (18 & 38 μg/kg) groups, respectively, was unexpected. The greatest mean increases in ETT of 107.9 and 133.1 seconds at 2 and 6 months, respectively, occurred in the high dose (24-48 μg/kg) IC group. The IV group exhibited significant mean increases in ETT of 93.4 seconds and 87.5 seconds, at 2 months and 6 months respectively, which was not predicted by the rat and pig animal models used herein. Overall, the persistence of the effect (increase in ETT) at six months and its magnitude for both the IC and IV groups was wholly unexpected.

The 66 human patients of the Phase I clinical trial described in Examples 1-3 herein were also evaluated using the Seattle Angina Questionnaire (SAQ). The SAQ is a validated, disease-specific, quality of life instrument which assesses the following five scales: 1) "exertional capacity" = limitation of physical activity; 2) "disease perception" = worry about MI; 3) "treatment satisfaction"; 4) "angina frequency" = number of episodes and sublingual nitroglycerin usage; and 5) "angina stability" = number of episodes with most strenuous physical activity. The possible range of scores for each of the five scales is 0 to 100 with the higher scores indicating a better quality of life. Typically, a mean change of 8 points or more between the mean baseline scores (*i.e.,* before treatment) and the post-treatment scores is recognized as being "clinically significant." However, in the present analysis, a dose was considered "effective" if the mean change in score from baseline increased by greater than 14 points. The reason that 14 was chosen (instead of 8) was to allow for the improvement that was seen in the placebo group at 2 months in a clinical trial of another growth factor--VEGF.

In performing the SAQ evaluation, the patients were categorized according to the same dosage groups that were evaluated for ETT, *i.e.*, 0.33 - 2.0 μg/kg IC (low) 6.0 - 12.0 μg/kg IC (mid); 24 - 48 μg/kg IC (high); and 18 and 36 μg/kg IV. The questionnaire was administered to subjects in each dosage group at baseline (prior to dosing), and at 2 months and 6 months after being administered a single unit dose composition of rFGF-2 in accordance with the method of the present invention.

According to Table 3, the change in score for angina stability increased relative to baseline at both 2 and 6 months for each group. The improvements in angina stability seen at 2 months after dosing (46.2, 32.1, 34.3 and 39.6) were significantly greater than those scores seen at 6 months (21.4, 16.7, 17.7 and 23.2). However, the scores found at both 2 months and 6 months after dosing showed that all dosages were found to be effective (> 14) in increasing angina stability. Moreover, the magnitude of the increases and their duration for 6 months were unexpected.

The third SAQ scale to be evaluated was "angina frequency." The data summarizing the angina frequency is presented in Table 4 herein. **Table 4. Angina Frequency (AF) – Change from Baseline**

FGF-2 Dose Group	*Change from Baseline at Two Months*	*Change from Baseline at Six Months*
0.33 to 2.0 μg/kg IC (N = 16) (low)	N = 14 27.9* (-10 to 80)	N = 7 12.9 (-40 to 50)
6.0 and 12 μg/kg IC (N = 8) (mid)	N = 7 32.9* (0 to 80)	N = 6 36.7 (-10 to 90)
24.0 to 48.0 μg/kg IC (N = 28) (high)	N = 27 28.9* (-40 to 80)	N = 24 25.8* (-30 to 80)
18.0 and 36.0 μg/kg IV (N = 14)	N = 12 20.0* (0 to 90)	N = 14 11.4 (-30 to 60)
ALL GROUPS (N = 66)	N = 60 27.3	N = 51 21.4

N = number of subjects; mean (range); * = $p \leq 0.05$

According to Table 4, the mean patient scores (27.9, 32.9, 28.9 and 20.0) for angina frequency increased at 2 months (relative to baseline) by an effective amount (> 14) for all dosage groups and for all modes of administration (IC or IV). The mean patient scores continued to increase at 6 months only for the mid dose (6.0 - 12.0 μg/kg) group, suggesting a peak effect at 2 months post-dosing. However, for the mid dose (6.0 - 12.0 μg/kg) and high dose (24.0 - 48.0 μg/kg) groups, the changes at 2 months and 6 months were similar, suggesting a persistent effect at 6 months on angina frequency. The third SAQ scale to be evaluated was "angina

Table 6. Disease Perception (DP) – Change from Baseline

Dose Group	*Change from Baseline at Two Months*	*Change from Baseline at Six Months*
0.33 to 2.0 μg/kg IC (N = 16) (low)	N = 14 29.2* (-8 to 58)	N = 7 26.2* (0 to 42)
6.0 and 12 μg/kg IC (N = 8) (mid)	N = 7 20.2* (-8 to 50)	N = 6 30.6* (0 to 58)
24.0 to 48.0 μg/kg IC (N = 28) (high)	N = 27 27.8* (-33 to 92)	N = 24 34.0* (-33 to 100)
18.0 and 36.0 μg/kg IV (N = 14)	N = 12 22.9* (-8 to 92)	N = 14 23.8* (-8 to 75)

N = number of subjects; mean (range); * = $p < 0.05$

Up to 60 of the human patients of the Phase I clinical trial described in Examples 1-3 herein were also evaluated using resting magnetic resonance imaging (MRI) scans of their heart. The resting MRI scans were performed on the patients at baseline, and at 1 month, 2 months and 6 months after dosing with a single unit dose composition of the present invention. The doses were considered "effective" based upon statistical significance ($p < 0.05$). The objective criteria assessed by the resting MRI scans are the following: (1) ejection fraction; (2) myocardial infarct extent (%); (3) normal wall thickening (4) normal wall motion (%); (5) target wall thickening (%); (6) target wall motion (%); (7) target wall area collateral extent (%); and (8) target area delayed arrival extent (%).

Based upon the resting MRI, no change in "ejection fraction" was observed at one month for any one group. The mean change from baseline for all groups (n = 33) at 1 month was an increase of 2.0% (p= 0.042). At two months, the mean change from baseline for the low dose IC group (n=13) was an increase of 8.1% (p=0.007); and for all groups (n=54), the mean change from baseline was an increase of 3.8% (p=0.001). At six months, the mean change from baseline for the high dose IC group (n=19) was 5.3% (p=0.023); for the IV group (n=3) was 11.1% (p=0.087); and for all groups (n=33) was 5.7% (p=0.001).

Thus, providing CAD patients with a single IC or IV infusion of rFGF-2 in accordance with the present invention provided the patients with a statistically significant physical improvement as objectively measured by MRI and other conventional criteria.

Pharmacokinetics and Metabolism

The molecular structure of FGF-2 contains a positively charged tail that is known to bind to proteoglycan chains (heparin and heparin-like structures) on cell surfaces and on the endothelial wall of the vasculature. *See* Moscatelli, *et al.*, *"Interaction of Basic Fibroblast Growth Factor with Extracellular Matrix and Receptors,"* Ann. NY Acad. Sci., **638**:177-181 (1981).

The kidneys and liver are the major organs for the elimination of rFGF-2. In particular, the kidneys have a protein cutoff of about 60 kD and thus retain serum albumin (MW 60 kD). However, FGF-2 (146 residues) has a molecular weight of about 16.5 kD. Accordingly, renal excretion is to be expected. In a radiolabelled biodistribution study of commercially available bovine FGF-2 (bFGF-2), both the liver and the kidney were shown to contain high counts of the radiolabelled bFGF-2 at 1 hour after IV or IC injection. In a published study, wherein another recombinant iodinated form of bFGF-2 was given to rats, the liver was identified as the major organ of elimination. Whalen *et al.*, *"The Fate of Intravenously Administered bFGF and the Effect of Heparin,"* Growth Factors, 1:157-164 (1989). It is also known that FGF-2 binds in the general circulation to α_2-macroglobulin and that this complex is internalized by receptors on the Kupffer cells. Whalen *et al.* (1989) and LaMarre *et al.*, *"Cytokine Binding and Clearance Properties of Proteinase-Activated Alpha-2-Macroglobulins,"* Lab. Invest., **65**:3-14 (1991). Labelled FGF-2 fragments were not found in the plasma, but they were found in the urine and corresponded in size to intracellular breakdown products.

In preclinical testing, we determined the pharmacokinetics of rFGF-2 (SEQ ID NO: 2) after intravenous (IV) and intracoronary (IC) administration in domestic Yorkshire pigs, and after IV administration dosing in Sprague Dawley ("SD") rats. The pig models demonstrated linear pharmacokinetics (0.65 μg/kg -

Figure 3 is a plot of individual human patient plasma clearance (CL) values (ml/min/kg) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion." Figure 3 shows the influence of timing of heparin administration on FGF-2 plasma clearance (CL). Although Figure 3 shows that administering heparin up to 100 minutes prior to rFGF-2 decreases FGF-2 clearance, the preferred time for administering heparin is from 0-30 minutes prior the rFGF-2 administration, wherein the effect of the heparin on decreasing FGF-2 clearance is greatest.

Figure 4 is a plot individual human patient rFGF-2 dose normalized area under curves (AUCs) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on rFGF-2 AUC. Figure 4 shows that the greatest AUC/dose was achieved when an effective amount of a glycosoaminoglycan, such as heparin, was preadministered within 30 minutes or less of IC rFGF-2 infusion, more preferably within 20 minutes or less of IC or IV rFGF-2 infusion. Typically, an effective amount of a glycosoaminoglycan is 10-80 U/kg heparin.

The mean pharmacokinetic parameters for rFGF-2 in humans as a function of dosage and mode of administration are summarized in Table 8 herein. Referring to Table 8, the T½ for FGF-2 in humans was determined to range from 2.2 $\pm$ 3.7 hours at low dose (0.33-2.0 μg/kg) IC to 7.0 $\pm$ 3.5 hours at a dose of 18-36 μg/kg IV; given the limitations of the assay, the terminal half-life is estimated at 5-7 hours for all groups. The clearances of FGF-2 ranged from 13.2 to 18.2 L/hour/70kg man. Finally, the steady state volume (V_{ss}) was determined to range from 11.3 $\pm$ 10.4 L/70kg man to 16.8 $\pm$ 10.7 L/70kg man.

Table 8. Mean rFGF-2 PK Parameters in Humans

FGF-2 Dose μg/kg	*N*	*Route*	*CL (L/hr/70kg)*	*$t_{½}$ (h)*	*V_{ss} (L/70kg)*
0.3 - 2	16	IC	18.2±13.4	2.2± 3.7	11.3±10.4
6 - 12	8	IC	13.2± 7.3	3.1± 2.5	12.1± 4.9
24 - 48	28	IC	14.7± 8.3	6.3± 1.8	16.8 ±10.7
18 - 36	14	IV	13.9± 7.9	7.0± 3.5	16.4± 8.6

the total infusion volume was increased to a maximum of 40 ml when proportionately higher absolute amounts of FGF-2 were administered to patients with higher body weights.

EXAMPLE 2

"Selection Criteria For Patients With Coronary Artery Disease For Treatment With rFGF-2"

The following selection criteria were applied to Phase I patients with coronary artery disease, whose activities were limited by coronary ischemia despite optimal medical management, and who were not candidates for approved revascularization therapies:

Inclusion criteria: Subject is eligible if:

- Male or female, greater than or equal to 18 years of age
- Diagnosis of coronary artery disease (CAD)
- Suboptimal candidates for approved revascularization procedures, *e.g.*, angioplasty, stents, coronary artery bypass graft (CABG) (or refuses those interventions)
- Able to exercise at least three minutes using a modified Bruce protocol and limited by coronary ischemia
- Inducible and reversible defect of at least 20% myocardium on pharmacologically stressed thallium sestamibi scan
- CBC, platelets, serum chemistry within clinically acceptable range for required cardiac catheterization
- Normal INR, or if anticoagulated with Coumadin, INR <2.0
- Willing and able to give written informed consent to participate in this study, including all required study procedures and follow-up visits

Exclusion criteria: Subject is not eligible if:

- Malignancy: any history of malignancy within past ten years, with the exception of curatively treated basal cell carcinoma.

- Any condition which makes the subject unsuitable for participation in this study in the opinion of the investigator, *e.g.*, psychosis, severe mental retardation, inability to communicate with study personnel, drug or alcohol abuse.

EXAMPLE 3

"Phase I Clinical Study on Recombinant FGF-2 (SEQ ID NO: 2) Administered to Humans"

The Phase I CAD trial of this example is an open label, dose escalation study of recombinant fibroblast growth factor-2 (rFGF-2) for safety, tolerability and pharmacokinetics. The study was conducted at two sites: Beth Israel Deaconess Hospital (Harvard) in Boston, MA and Emory University Hospital in Atlanta, GA. Enrollment is complete. Subjects were treated with a single infusion of rFGF-2 on Day 1 and followed for 360 days; follow-up is not yet complete in some subjects.

The subject population consists of patients with advanced CAD who are exercise limited by coronary ischemia and are considered suboptimal candidates for (or do not want to undergo) one of the established revascularization procedures (*e.g.*, CABG, angioplasty -- with or without stent). The major exclusion criteria were history or suspicion of malignancy, uncompensated heart failure or left ventricular ejection fraction <20%, renal insufficiency or proteinuria, and various ocular conditions (e.g., proliferative diabetic retinopathy, severe non-proliferative retinopathy).

Sixty-six subjects have received rFGF-2 of SEQ ID NO: 2 in this trial: fifty-two received the rFGF-2 as an IC infusion and fourteen received it as an IV infusion. Each subject was observed in the hospital for at least twenty-four hours, and then followed as an outpatient for 360 days with follow-up visits (Days 15, 29, 57, 180 and 360). At least four subjects were studied at each dose; if no subject experienced dose-limiting toxicity as defined by the protocol within six days, the dose was escalated. The drug was administered as a single 20 minute infusion divided between two major sources of coronary blood supply (IC), using standard techniques for positioning a catheter into the patient's coronary artery (such as

Preliminary Results

The results presented here are unaudited and are based on a third interim analysis for sixty-six subjects with six months follow - up for all groups (1-10) and the serious adverse events (SAE) report of 29 July 1999 from Chiron Drug Safety. Data collection for the last visit (Day 360) and final analysis is in progress.

The starting dose of 0.33 μg/kg IC was escalated over eight sequential groups to 48 μg/kg IC, at which dose 2 of ten subjects experienced dose-limiting toxicity (hypotension) as defined by the protocol. Hypotension was manageable with fluids alone in all subjects (no vasopressors or assistive devices). At 36 μg/kg IC, only 1 of 10 subjects had dose-limiting toxicity which defined this dose as the maximally tolerated dose (MTD). Two additional groups were studied by IV infusion; four subjects of half the MTD (18 μg/kg) and ten subjects at the MTD (36 μg/kg).

Hypotension was dose-limiting in humans, as predicted by the animal model in Yorkshire pigs. However, 36.0 μg/kg rFGF-2 IC was tolerated in humans; whereas in pigs, 20.0 μg/kg rFGF-2 IC caused profound hypotension in one of two animals. Better tolerability in humans was attributed to aggressive fluid management and absence of general anesthesia.

As of 29 July 1999, thirty-three serious adverse events (SAEs) have occurred in 24/66 subjects, but were not dose-related. Fifteen (15) SAEs were considered at least possibly related to rFGF-2; whenever there was a difference between relatedness assigned by the investigator and the medical monitor, the more conservative relationship was assigned. SAE's were multiple in five subjects: 01103 (0.33 μg/kg IC), 01106 (0.65 μg/kg IC),. 01113 (2.0μg/kg IC), 01137 (36.0 μg/kg IV), 02101 (0.65 μg/kg IC).

The most frequent treatment-emergent adverse events (AEs) on Day 1 were transient systolic hypotension and transient bradycardia. The hypotension was dose-dependent and occurred more frequently at doses greater than or equal to (≥) 24 μg/kg IC; bradycardia was not dose-dependent. Other adverse events (AEs) which were deemed at least possibly related and appeared dose-related occurred within the first several days or week post infusion and included chest pain, shortness

The human patients in this study that were treated with a single IC or IV infusion of rFGF-2 of SEQ ID NO: 2 exhibited a mean increase in ETT of 1.5 to 2 minutes. *See* Table 1. This is especially significant because an increase in ETT of greater than (>) 30 seconds is considered significant and a benchmark for evaluating alternative therapies, such as angioplasty. The angina frequency and quality of life, as measured by SAQ, showed a significant improvement at 2 months in all five subscales for the 66 patients (n=66) tested. See Tables 26. In Tables 2-6, a mean change of 14 or more was considered "clinically significant."

When 33 human CAD patients were assessed by resting cardiac magnetic resonance imaging (MRI) at baseline, and at 1, 2, and 6 months after receiving a single unit dose composition of the present invention by IC or IV routes, a highly statistically significant increase was observed in target wall thickening, target wall motion and target area collateral extent; a highly statistically significant decrease was observed in target area delayed arrival extent; and no statistically significant changes were observed in normal wall motion, normal wall thickening or myocardial infarct extent.

In addition to the above criterion (*i.e.*, ETT SAQ, MRI), a treatment is considered very successful if the angiogenic effects last at least six months. In the present Phase I study, the unexpectedly superior angiogenic effects were observed to last up to 6 months in some patients in all dosage groups. Based upon the results already obtained, it is expected that the angiogenic effects may last twelve months or more but do last at least six months in the patients, at which time the procedure could be repeated, if necessary.

EXAMPLE 4

"Proposed Phase II Clinical Study On rFGF-2 (SEQ ID NO: 2) Administered to Humans to Treat Coronary Artery Disease"

The Phase II clinical trial of rFGF-2 for treating human patients for coronary artery disease is performed as a double blind/placebo controlled study having four arms: placebo, 0.3 μg/kg, 3.0 μg/kg, and 30 μg/kg administered once IC. This study is ongoing and results are not yet available.

CLAIMS

What is claimed is:

1. A unit dose composition for inducing angiogenesis in a human, comprising about .008 mg to about 7.2 mg of FGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier.

2 The unit dose composition of claim 1, comprising 0.3 mg to 3.5 mg of FGF-2, or an angiogenically active fragment or mutein thereof.

3. The unit dose composition of claim 1, wherein said FGF-2 has the amino acid sequence of SEQ ID NO: 2.

4. The unit dose composition of claim 3, comprising 0.3 mg to 3.5 mg of an FGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier.

5. The unit dose composition of claim 3, comprising about .008 mg to about 7.2 mg of said angiogenically active mutein of said FGF-2 of SEQ ID NO: 2 in a pharmaceutically acceptable carrier.

6. The unit dose composition of claim 5, comprising 0.3 mg to 3.5 mg of said angiogenically active mutein of said FGF-2 of SEQ ID NO: 2 in a pharmaceutically acceptable carrier.

13. The method of claim 12, wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof is administered to one or more coronary vessels.

14. The method of claim 13, wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof is about 24 μg/kg to 48 μg/kg.

15. The method of claim 12 wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or said angiogenically active fragment or mutein thereof is administered to a peripheral vein.

16. The method of claim 15, wherein said therapeutically effective amount of a recombinant FGF-2 of SEQ ID NO: 2 or said angiogenically active fragment or mutein thereof is about 18 μg/kg to 36 μg/kg.

17. A method for treating a human patient for coronary artery disease comprising, administering a single unit dose of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in a human patient in need of treatment for coronary artery disease, said unit dose comprising from about .008 mg to 7.2 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof.

25. The method of claim 19, further comprising the step of administering 10 U/kg to 80 U/kg of heparin to said patient IV or IC about 0 to 30 minutes prior to administering said unit dose.

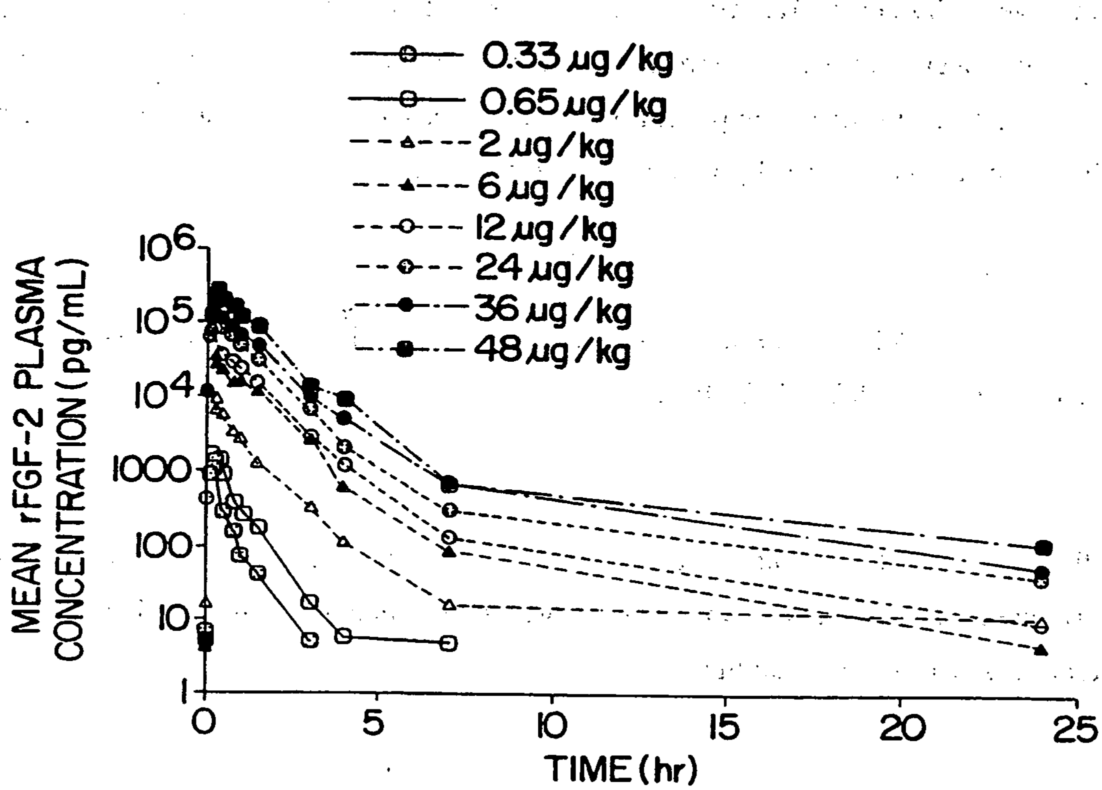
26. A method for inducing angiogenesis in a heart of a human patient comprising, administering a single unit dose of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in a human patient in need of treatment for coronary artery disease, said unit dose comprising from about .008 mg to 7.2 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof.

27. The method of claim 26, wherein said FGF-2 has the amino acid sequence of SEQ ID NO: 2.

28. The method of claim 27 wherein said single unit dose produces an improvement in one or more clinical endpoints in said human patient that lasts at least four months.

29. The method of claim 28, wherein said single unit dose produces an improvement in one or more clinical endpoints in said human patient that lasts 6 months.

30. A method for treating a human patient for a myocardial infarction comprising, administering a single unit dose of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in said human patient, said unit dose comprising from about


0.33 µg/kg
0.65 µg/kg
2 µg/kg
6 µg/kg
12 µg/kg
24 µg/kg
36 µg/kg
48 µg/kg
MEAN rFGF-2 PLASMA CONCENTRATION (pg/mL)
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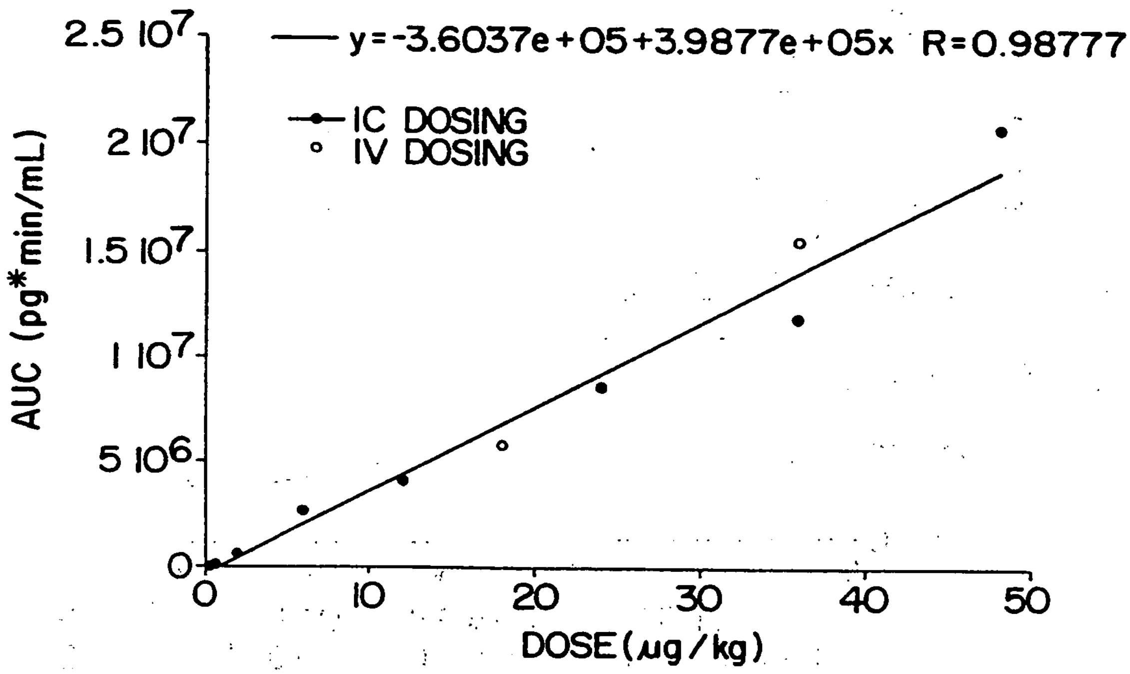
10^5
10^4
1000
100
10
1
0
5
10
15
20
25
TIME (hr)

FIG. 1A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IV DOSING
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1 10^7
5 10^6
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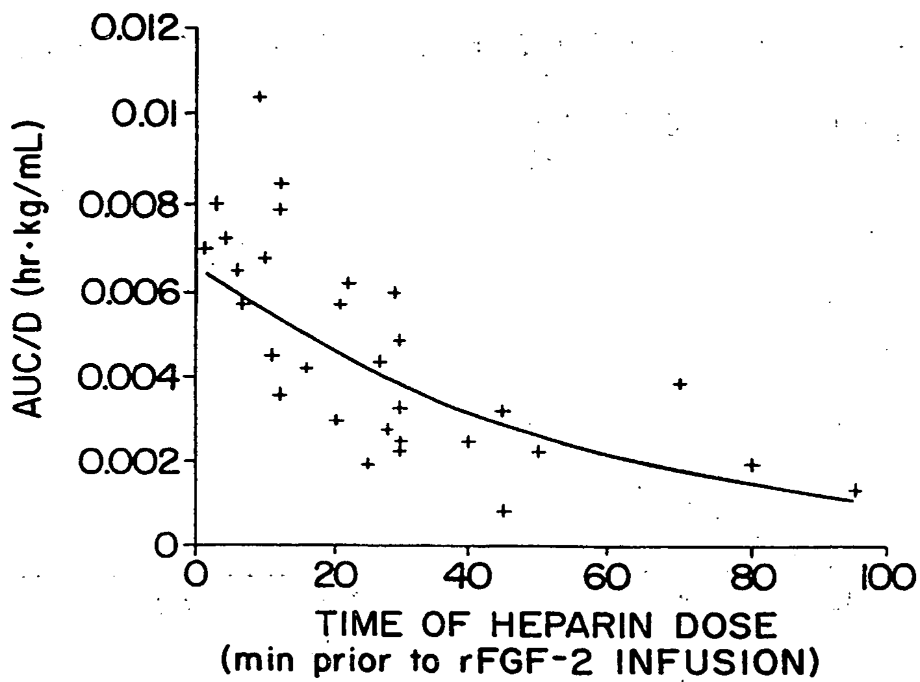
30
40
50
DOSE (μg/kg)

FIG. 2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0.01
0.008
0.006
0.004
0.002
0
AUC/D (hr·kg/mL)
0
20
40
60
80
100
TIME OF HEPARIN DOSE
(min prior to rFGF-2 INFUSION)

FIG. 4

cga cta gaa tcc aac aac tat aac acc tat cga tcc cga aaa tat tcc 336
Arg Leu Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Ser
100 105 110

tcc tgg tat gta gcc cta aaa cga acc ggg caa tat aaa cta ggg cca 384
Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Pro
115 120 125

aaa acc ggg cca ggg caa aaa gcc atc cta ttc cta cca atg tcc gcc 432
Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala
130 135 140

aaa tcc taag 442
Lys Ser
145

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35 40 45

Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser
50 55 60

Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly
65 70 75 80

Arg Leu Leu Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu
85 90 95

Arg Leu Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Ser
100 105 110

Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Pro
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Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala
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Lys Ser
145

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/19770

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/18 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SELLKE FRANK W ET AL: "Therapeutic angiogenesis with basic fibroblast growth factor: Technique and early results." ANNALS OF THORACIC SURGERY JUNE, 1998, vol. 65, no. 6, June 1998 (1998-06), pages 1540-1544, XP000891950 ISSN: 0003-4975 the whole document -/--	1,9-34

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2000	03/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT	International application No. PCT/US 99/19770

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.